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|--|---|---|
| Form PTO 1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE<br>(REV 5-93)  |   | ATTORNEY'S DOCKET NUMBER<br>B45168                              |
| TRANSMITTAL LETTER TO THE UNITED STATES<br>DESIGNATED / ELECTED OFFICE (DO/EO/US)<br>CONCERNING A FILING UNDER 35 U.S.C. 371 |   | U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)<br>09/868604 |
| INTERNATIONAL APPLICATION NO<br>PCT/EP99/10297   | INTERNATIONAL FILING DATE<br>21 December 1999 | PRIORITY DATE CLAIMED<br>21 December 1998                       |
| TITLE OF INVENTION<br>VACCINE  |   |   |
| APPLICANT(S) FOR DO/EO/US<br>Alex BOLLEN, Alain FAUCONNIER, and Edmond GODFROID  |   |   |

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. to 16. below concern other document(s) or information included:**

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98; and Form PTO-1449.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☒ Please amend the specification by inserting before the first line the sentence: This is a 371 of International Application PCT/EP99/10297, filed December 21, 1999, which claims benefit from the following Provisional Application: GB 9828217.1 filed 21 December 1998.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☒ An Abstract on a separate sheet of paper.
19. ☒ Other items or information: Sequence Listing, Statement to Support, Diskette

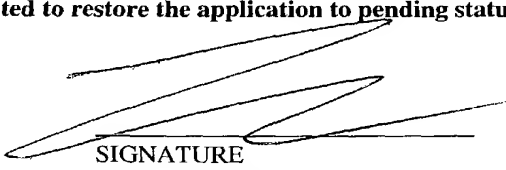
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JC18 Rec'd PCT/PTO 2 0 JUN 2001

|   |                  |   |                     |                               |    |
|---|------------------|---|---------------------|-------------------------------|----|
| US APPLICATION NO. (if known see 37 CFR 1.50)<br><b>09/868604</b>   |                  | INTERNATIONAL APPLICATION NO.<br>PCT/EP99/10297 |                     | ATTORNEYS DOCKET NO<br>B45168 |    |
| 20. [X] The following fees are submitted:   |                  |   |                     | CALCULATIONS PTO USE ONLY     |    |
| <b>Basic National Fee (37 C.F.R. 1.492(a)(1)-(5)):</b>  |                  |   |                     | <b>\$710.00</b>               |    |
| Search Report has been prepared by the EPO or JPO .....\$860.00   |                  |   |                     |                               |    |
| International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) .....\$690.00  |                  |   |                     |                               |    |
| No International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....\$710.00   |                  |   |                     |                               |    |
| Neither International Preliminary Examination Fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$1,000.00   |                  |   |                     |                               |    |
| International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....\$100.00  |                  |   |                     |                               |    |
| <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>   |                  |   |                     | <b>\$710.00</b>               |    |
| Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).       |                  |   |                     | <b>\$0.00</b>                 |    |
| Claims  | Number Filed     | Number Extra                                    | Rate                |                               |    |
| Total claims  | <b>99 - 20 =</b> | <b>79</b>                                       | <b>79 x \$18.00</b> | <b>\$1422.00</b>              |    |
| Independent claims  | <b>10 - 3 =</b>  | <b>7</b>  | <b>7 x \$80.00</b>  | <b>\$560.00</b>               |    |
| Multiple dependent claims (if applicable)   |                  |   | <b>+ \$270.00</b>   | <b>\$270.00</b>               |    |
| <b>TOTAL OF ABOVE CALCULATIONS =</b>  |                  |   |                     | <b>\$2252.00</b>              |    |
| Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).  |                  |   |                     | <b>\$</b>                     |    |
| <b>SUBTOTAL =</b>   |                  |   |                     | <b>\$2962.00</b>              |    |
| Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)) + |                  |   |                     | <b>\$</b>                     |    |
| <b>TOTAL NATIONAL FEE =</b>   |                  |   |                     | <b>\$2962.00</b>              |    |
|   |                  |   |                     | Amount to be refunded         | \$ |
|   |                  |   |                     | charged                       | \$ |

- a. ☐ A check in the amount of \$\_\_\_\_\_ to cover the above fees is enclosed.
- b. ☒ Please charge my Deposit Account No. 19-2570 in the amount of **\$2962.00** to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-2570. A duplicate copy of this sheet is enclosed.
- d. ☒ General Authorization to charge any and all fees under 37 CFR 1.16 or 1.17, including petitions for extension of time relating to this application (37 CFR 1.136 (a)(3)).

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:  
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SIGNATURE  
Zoltan Kerekes  
NAME  
38,938  
REGISTRATION NO.

09/868604

09/868604

**"EXPRESS MAIL CERTIFICATE"**  
**"EXPRESS MAIL" MAILING LABEL NUMBER EL73784907/US**  
**DATE OF DEPOSIT: 20 June 2001**

IC18 Rec'd PCT/PTO 2 0 JUN 2001

Attorney Docket No. B45168

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Bollen, et al. 20 June 2001

Int'l. App. No.: PCT/EP99/10297 Group Art Unit: Not Yet Assigned

Int'l. Filing Date: 21 December 1999 Examiner: Not Yet Assigned

For: VACCINE

Assistant Commissioner of Patents  
 Box: PCT  
 Washington, D.C. 20231

**PRELIMINARY AMENDMENT**

Preliminary to the examination of this application, Applicants respectfully request consideration and entry of the following Preliminary Amendment.

Applicants are submitting herewith a new Statement to Support Filing and Submission in Accordance with 37 CFR §§ 1.821 Through 1.825, which includes three corrected sheets (pages 111, 113, and 114) pursuant to 37 CFR §§ 1.825. In addition, Applicants are submitting the complete Sequence Listing on computer diskette.

**IN THE SPECIFICATION**

Please amend Table 3 on page 38 as follows:

**Table 3**

| Names   | Coding sequence<br>from/to (with<br>reference to Fig. 5) | Coding<br>DNA strand | SEQ ID<br>NO: | Homologous genes (from<br><i>Yersinia</i> , unless otherwise<br>specified) |
|---|--|----------------------|---------------|--|
| Class II ORFs which putatively code for effector proteins |  |                      |               |  |
| <i>bopN</i>   | 11906/13003  | complement           | 41            | <i>YopN</i> (= <i>lcrE</i> )   |
| <i>orf1</i>   | 6160/6747  | direct               | 43            | None   |
| <i>orf2</i>   | 10752/11120  | complement           | 45            | None   |

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|              |             |            |    |  |
|--------------|-------------|------------|----|--|
| <i>orf3</i>  | 11117/11527 | complement | 47 | None   |
| <i>orf4</i>  | 11532/11909 | complement | 49 | None   |
| <i>orf5</i>  | 13002/13784 | direct     | 51 | None   |
| <i>orf6</i>  | 13806/14081 | direct     | 53 | None   |
| <i>orf7</i>  | 14630/15571 | direct     | 55 | None   |
| <i>orf8</i>  | 15601/16803 | direct     | 57 | None   |
| <i>orf9</i>  | 16827/17288 | direct     | 59 | <i>BcrH</i>                                      |
| <i>orf10</i> | 17293/17814 | direct     | 61 | <i>pcr4</i><br>( <i>Pseudomonas aeruginosa</i> ) |
| <i>orf11</i> | 29412/29591 | complement | 63 | None   |
| <i>orf12</i> | 29555/30529 | complement | 65 | None   |
| <i>orf13</i> | 30631/31776 | direct     | 67 | None   |
| <i>orf14</i> | 31818/33005 | complement | 69 | None   |
| <i>orf15</i> | 32370/33014 | direct     | 71 | None   |

IN THE CLAIMS:

Please cancel claims 1-29.

Please add new claims 30-78.

30. An isolated polypeptide comprising an amino acid sequence which has at least 75% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72 over its entire length.

31. The polypeptide as claimed in claim 30 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72.

32. An isolated polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.



33. An isolated polypeptide comprising a fragment of at least 7 consecutive amino acids of the polypeptide as claimed in any one of claims 30 to 32, wherein the fragment comprises an epitope.
34. The polypeptide of claim 33, wherein the fragment is immunogenic.
35. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 75% identity to the amino acid sequence of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.
36. An isolated polynucleotide comprising a nucleotide sequence that has at least 75% identity to a nucleotide sequence, encoding a polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.
37. An isolated polynucleotide which comprises a nucleotide sequence which has at least 75% identity to that of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.
38. The isolated polynucleotide as claimed in claim 35 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.
39. The isolated polynucleotide as claimed in claim 36 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.
40. The isolated polynucleotide as claimed in claim 37 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

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41. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

42. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71.

43. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 or a fragment thereof.

44. An expression vector comprising an isolated polynucleotide according to any one of claims 35-43.

45. A recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 35-43.

46. A host cell comprising the expression vector of claim 44 or a subcellular fraction or a membrane of said host cell.

47. A process for producing the polypeptide of claim 30 comprising the steps of culturing a host cell of claim 46 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

48. A process for expressing a polynucleotide of any one of claims 35-43 comprising transforming a host cell with an expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

49. A vaccine composition comprising an effective amount of the polypeptide of claim 30 and a pharmaceutically acceptable carrier.

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50. A vaccine composition comprising an effective amount of the polypeptide of claim 31 and a pharmaceutically acceptable carrier.
51. A vaccine composition comprising an effective amount of the polypeptide of claim 32 and a pharmaceutically acceptable carrier.
52. A vaccine composition comprising an effective amount of the polypeptide of claim 33 and a pharmaceutically acceptable carrier.
53. A vaccine composition comprising an effective amount of the polypeptide of claim 34 and a pharmaceutically acceptable carrier.
54. The vaccine composition of claim 49, wherein the polypeptide has an amino acid sequence selected from the group consisting of: SEQ ID NO:42, 46, 48, 50, 52, 54, 56, 58, 60 and 62.
55. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 35 to 43 and a pharmaceutically acceptable carrier.
56. The vaccine composition according to any one of claims 49-55, wherein said composition comprises at least one other *Bordetella pertussis* antigen.
57. An antibody immunospecific for the amino acid sequence of claim 30 or 31.
58. An antibody immunospecific for the polypeptide of claim 32.
59. An antibody immunospecific for the fragment of claim 33.
60. An antibody immunospecific for the fragment of claim 34.
61. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 30, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

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62. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 31, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.
63. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 32, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.
64. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 33, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.
65. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 34, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.
66. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 30 and a suitable pharmaceutical carrier.
67. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 31 and a suitable pharmaceutical carrier.
68. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 32 and a suitable pharmaceutical carrier.
69. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 33 and a suitable pharmaceutical carrier.

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70. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 34 and a suitable pharmaceutical carrier.
71. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polynucleotide of claims 35-43.
72. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 30.
73. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 31.
74. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 32.
75. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 33.
76. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 34.
77. A method of identifying virulence genes from a pathogenicity island containing a type III secretion system from pathogenic strains of bacteria, comprising:
  - designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;
  - amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers of *lcrD*-like genes present in said pathogenic strain of bacteria;
  - sequencing the *lcrD*-like gene;
  - determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes; and

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if a virulence-associated member, sequencing the entire pathogenicity island, and identifying genes within this sequence.

78. A method of determining whether a particular bacterial strain harbours a type III secretion system involved in pathogenicity, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers to determine the presence of any *lcrD*-like genes in said bacterial strain;

if amplified successfully, sequencing the *lcrD*-like gene; and

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes.

#### REMARKS

The above-identified application is being entered into the National Phase from PCT application no. PCT/EP99/10297.

#### Specification and Sequence Listing

An inadvertant error on page 38 (Table 3) which recites position 31773 as the end of the open reading frame for *orf14* has been corrected to show position 31818. As set forth below, correcting this error is obvious.

Applicants respectfully request amendment of SEQ ID NO:69 and SEQ ID NO:70 as set forth on substitute sheets 111, 113, and 114 submitted herewith pursuant to 37 CFR §§ 1.825.

In the original submission of the Sequence Listings, the Sequence Listing program completely ignored the stop codon at position 396. Although the sequence shown in Fig. 5 is correct, Table 3 states that *orf14* is encoded in the complementary strand from position 31773-33005. Although position 33005 indicates the correct start codon for this open reading frame, the end of the open reading frame is incorrectly stated. It should actually be at position 31818 – where the first stop codon (at position 396) is encountered.

Correcting this error is obvious. Given a properly stated start codon, and a correct DNA sequence, anyone would realize that the open reading frame MUST end where the first, in-frame,

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stop codon in the sequence is encountered. This is obviously at position 396, and was clearly the intention of the Applicants at the time of filing the international application.

Three replacement sheets are provided as required under 37 CFR §§ 1.825. The error on page 111 has been rectified to indicate the number of nucleotides in SEQ ID NO:69 as being 1188. The error on page 113 has been rectified to indicate that the open reading frame does not extend past the stop codon at position 396. In addition, the number of amino acids in SEQ ID NO:70 has been rectified as being 395. The error on page 114 has been rectified to indicate the last amino acid in the open reading frame as being His395.

The corrected sequence listing for SEQ ID NO:69 and 70 does not go beyond the disclosure apparent to anyone from Fig. 5 and Table 3 of the International Application as filed.

The complete sequence listing is provided on a computer diskette. It is identical to the original written sequence listing in conjunction with the aforementioned corrections to SEQ ID NO: 69 and 70.

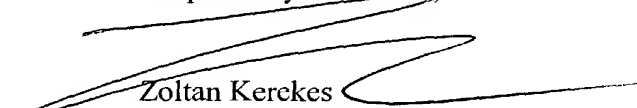
#### Claims

Claims 1-29 were cancelled. New claims 30-78 were added for the following reason: to put the claims in conformity with U.S. practice.

No new matter has been introduced.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with Markings to Show Changes Made**". Applicants respectfully request that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

  
Zoltan Kerekes  
Attorney for Applicants  
Registration No. 38,938

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**VERSION WITH MARKINGS TO SHOW CHANGES**

**IN THE SPECIFICATION:**

**Table 3 appearing on page 38 has been amended as follows:**

**Table 3**

| Names   | Coding sequence<br>from/to (with<br>reference to Fig. 5) | Coding<br>DNA strand | SEQ ID<br>NO: | Homologous genes (from<br><i>Yersinia</i> , unless otherwise<br>specified) |
|---|--|----------------------|---------------|--|
| Class II ORFs which putatively code for effector proteins |  |                      |               |  |
| <i>bopN</i>   | 11906/13003  | complement           | 41            | <i>YopN</i> (= <i>lcrE</i> )   |
| <i>orf1</i>   | 6160/6747  | direct               | 43            | None   |
| <i>orf2</i>   | 10752/11120  | complement           | 45            | None   |
| <i>orf3</i>   | 11117/11527  | complement           | 47            | None   |
| <i>orf4</i>   | 11532/11909  | complement           | 49            | None   |
| <i>orf5</i>   | 13002/13784  | direct               | 51            | None   |
| <i>orf6</i>   | 13806/14081  | direct               | 53            | None   |
| <i>orf7</i>   | 14630/15571  | direct               | 55            | None   |
| <i>orf8</i>   | 15601/16803  | direct               | 57            | None   |
| <i>orf9</i>   | 16827/17288  | direct               | 59            | <i>BcrH</i>  |
| <i>orf10</i>  | 17293/17814  | direct               | 61            | <i>pcr4</i><br>( <i>Pseudomonas aeruginosa</i> )                           |
| <i>orf11</i>  | 29412/29591  | complement           | 63            | None   |
| <i>orf12</i>  | 29555/30529  | complement           | 65            | None   |
| <i>orf13</i>  | 30631/31776  | direct               | 67            | None   |
| <i>orf14</i>  | [31773]31818/330<br>05                                   | complement           | 69            | None   |
| <i>orf15</i>  | 32370/33014  | direct               | 71            | None   |



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**IN THE SEQUENCE LISTING:**

**IN THE CLAIMS:**

**Claims 1-29 have been cancelled. New claims 30-78 have been added as follows:**

30. An isolated polypeptide comprising an amino acid sequence which has at least 75% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72 over its entire length.
31. The polypeptide as claimed in claim 30 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72.
32. An isolated polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.
33. An isolated polypeptide comprising a fragment of at least 7 consecutive amino acids of the polypeptide as claimed in any one of claims 30 to 32, wherein the fragment comprises an epitope.
34. The polypeptide of claim 33, wherein the fragment is immunogenic.
35. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 75% identity to the amino acid sequence of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.
36. An isolated polynucleotide comprising a nucleotide sequence that has at least 75% identity to a nucleotide sequence, encoding a polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

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37. An isolated polynucleotide which comprises a nucleotide sequence which has at least 75% identity to that of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

38. The isolated polynucleotide as claimed in claim 35 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

39. The isolated polynucleotide as claimed in claim 36 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

40. The isolated polynucleotide as claimed in claim 37 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

41. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

42. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71.

43. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 or a fragment thereof.

44. An expression vector comprising an isolated polynucleotide according to any one of claims 35-43.

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45. A recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 35-43.

46. A host cell comprising the expression vector of claim 44 or a subcellular fraction or a membrane of said host cell.

47. A process for producing the polypeptide of claim 30 comprising the steps of culturing a host cell of claim 46 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

48. A process for expressing a polynucleotide of any one of claims 35-43 comprising transforming a host cell with an expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

49. A vaccine composition comprising an effective amount of the polypeptide of claim 30 and a pharmaceutically acceptable carrier.

50. A vaccine composition comprising an effective amount of the polypeptide of claim 31 and a pharmaceutically acceptable carrier.

51. A vaccine composition comprising an effective amount of the polypeptide of claim 32 and a pharmaceutically acceptable carrier.

52. A vaccine composition comprising an effective amount of the polypeptide of claim 33 and a pharmaceutically acceptable carrier.

53. A vaccine composition comprising an effective amount of the polypeptide of claim 34 and a pharmaceutically acceptable carrier.

54. The vaccine composition of claim 49, wherein the polypeptide has an amino acid sequence selected from the group consisting of: SEQ ID NO:42, 46, 48, 50, 52, 54, 56, 58, 60 and 62.

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55. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 35 to 43 and a pharmaceutically acceptable carrier.

56. The vaccine composition according to any one of claims 49-55, wherein said composition comprises at least one other *Bordetella pertussis* antigen.

57. An antibody immunospecific for the amino acid sequence of claim 30 or 31.

58. An antibody immunospecific for the polypeptide of claim 32.

59. An antibody immunospecific for the fragment of claim 33.

60. An antibody immunospecific for the fragment of claim 34.

61. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 30, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

62. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 31, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

63. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 32, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

64. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 33, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

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65. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed claim 34, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

66. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 30 and a suitable pharmaceutical carrier.

67. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 31 and a suitable pharmaceutical carrier.

68. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 32 and a suitable pharmaceutical carrier.

69. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 33 and a suitable pharmaceutical carrier.

70. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claim 34 and a suitable pharmaceutical carrier.

71. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polynucleotide of claims 35-43.

72. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 30.

73. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polypeptide of claim 31.



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determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes.

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#### Abstract

This invention relates to a general method for detecting pathogenic strains of bacteria which harbour a type III secretion system. More particularly, this invention relates to the methods as applied to the pathogen *Bordetella pertussis*. Furthermore, the invention relates to newly identified polynucleotides within these regions, virulent polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly the polynucleotides and polypeptides of the present invention relate to the virulent effector proteins associated with the type III secretion system of *Bordetella pertussis*, which are particularly suitable for vaccine purposes.



VACCINE**FIELD OF INVENTION**

This invention relates to a general method for detecting pathogenic strains of bacteria that harbour a type III secretion system, and characterising regions of the chromosome of said strain where virulence genes reside. More particularly, this invention relates to the method as applied to the pathogen *Bordetella pertussis*. Furthermore, the invention relates to newly identified polynucleotides within these regions, virulent polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production.

**BACKGROUND OF THE INVENTION****Type III secretion systems:**

Pathogenic bacteria invade many different niches in a broad host range and cause a wide variety of syndromes. It is due to this fact that it was believed previously that each disease might be induced by a distinct molecular mechanism. However, the spectrum of such mechanisms is not as broad as first imagined; rather, bacteria exploit a number of common molecular tools to achieve a range of goals. Among these tools are type III secretion systems, which provide a means for bacteria to target virulence factors directly at host cells. These factors then tamper with host cell functions to the pathogens' benefit.

The type III export system is responsible for secretion of *Salmonella* and *Shigella* invasion and virulence factors, Enteropathogenic *Escherichia coli* (EPEC) signal transduction molecules, virulence factors in several plant pathogens (for instance *Xanthomonas campestris* pv. *vesicatoria* [Fenselau *et al.*, 1992]) and Yops proteins in *Yersinia*. Yops export mechanism has been the most intensively investigated type III secretion apparatus (see for instance: Allaoui *et al.*, 1994; Bergman *et al.*, 1994). In this system, more than 20 different Ysc/Lcr proteins, all encoded by the virulence plasmid pYV, are presumed to compose a secretion channel spanning the *Yersinia* cell envelope.

Besides these elements involved in the secretion machinery, the pYV plasmid codes for the Yops proteins which are the secreted substrates and appear as the actual effectors of virulence.

5 Comparative studies of type III secretion systems originating from different species reveal that the components of the secretion machinery are conserved (Gygi *et al.*, 1995; Bogdanove *et al.*, 1996). In addition, homologs have been found in determinants which take part in flagellar assembly, indicating that this secretion pathway may be involved in surface organelle biosynthesis (Ramakrishnan *et al.*, 1991).

10 In contrast, however, the secreted substrates share no similarities, except in few cases. Therefore, the abandoned concept of a distinct molecular mechanism corresponding to each disease could reappear at the level of effector proteins.

### 15 Pathogenicity island

Pathogenicity islands have emerged as a novel theme in the field of bacterial virulence. Although they can comprise type III secretion systems they do not exclusively  
20 do so.

Early in the search for virulence genes, it was observed that many of these genes resided on plasmids. However, numerous virulence genes were also found on the chromosome. Surprisingly, the chromosomal virulence genes are also often clustered in  
25 functionally related groups. Such groups of virulence genes gave rise to the concept of pathogenicity islands (Pais) which can be defined as compact, distinct genetic units carrying virulence genes. These units, often flanked by direct repeats, occupy large chromosomal regions (often > 30 kb) and are present in pathogenic strains, whilst being absent or sporadically distributed in less-pathogenic (or non-pathogenic) strains of a  
30 bacterial species. These DNA segments are frequently associated with tRNA genes

and/or insertion sequence (IS) elements at their boundaries. In addition, their G+C content often differs from that of host bacterial DNA, suggesting a foreign origin.

Pathogenicity islands have been discovered in an increasing number of bacterial pathogens, including different categories of *E. coli*, *Salmonella typhimurium*, *Yersinia* spp, *Helicobacter pylori*, *Vibrio cholera* etc.

The first intensively studied pathogenicity islands were Pai I and Pai II, which encode the haemolysin determinants of uropathogenic *E. coli*. These two Pairs, are flanked by direct repeats and can be deleted from the chromosome at frequencies of  $10^{-4}$ , resulting in non-virulent mutant strains. Another pathogenicity island of 35 kb has recently been identified on the chromosome of enteropathogenic *E. coli* (EPEC) and was found to encode all known determinants involved in the so-called "attaching and effacing" (AE) lesion formation. This region was therefore referred to as "locus of enterocyte effacing" (LEE). Despite the fact that uropathogenic and enteropathogenic *E. coli* cause completely different infectious diseases, Pai I of the uropathogenic strains and the LEE locus of EPEC are inserted at exactly the same positions into the *E. coli* chromosome.

While some authors support a definition of pathogenicity islands which necessarily includes its chromosomal location, others have extended the concept to blocks of virulence genes, regardless of their location in chromosomes, plasmids or phages. The fact that, on one hand, phages and plasmids can easily insert into and excise from the chromosome and, on the other, that cryptic origins of plasmid replication, or phage related sequences were detected in Pairs, prompted the latter and less restrictive definition.

The pathogenicity islands (PAIs) which code for a type III secretion system encompass genes that divide into two classes, I and II. Class I encompasses the genes coding for the secretion machinery components and their regulators of expression, class

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II encompasses the genes encoding secreted effector proteins. Both *Yersinia lcrD* and *yscU* belong to class I. The precise functions of class I determinants is not well understood. Although it is sometimes not straightforward to make a clear distinction between class I and class II components, genes of class I can be identified as being present in many different species, and a comparison of their respective gene sequences indicate that equivalent genes share a significant (*yscI*, *yscO*) or even high level (*lcrD*, *yscU*, *yscN*) of sequence similarity (Hueck, 1998).

The second class of genes (class II) codes for proteins which constitute the substrate secreted by the translocon. These proteins appear as the actual effectors of virulence and are referred to as target proteins, virulence effector proteins or, simply, effectors. In contrast to the situation prevailing in class I gene products, the effectors share no, or very weak, similarities between species. Effector proteins are those which present the best biological, vaccine and diagnostic potentialities.

The inventors have discovered that the clustering of class I and class II genes inside a single pathogenicity island, offers the opportunity of conveniently finding and characterising unknown class II genes by targeting class I genes which can be identified using a known sequence of one of their numerous orthologues.

### ***Bordetella pertussis***

Whooping cough is a disease caused by infection by *Bordetella pertussis*, and is a serious and debilitating human disease particularly in young children. Although whole cell and acellular vaccines are available that are effective against the disease, there remains a need for the identification of further highly purified pertussis proteins that could be used in a more efficacious pertussis vaccine.

Although many pertussis virulence associated factors are known such as pertussis toxin, filamentous haemagglutinin, pertactin, which have been included in various acellular vaccines, there is no convenient genetic method for identifying further virulence factors using the pertussis genome (short of laboriously sequencing the whole genome).

5 Although class I type III secretion system virulence genes have recently been shown to exist in *B. bronchiseptica* and *B. pertussis* (Yuk *et al.*, 1998), there has been no complete analysis of a pathogenicity island in *Bordetella*, and the identity and characterisation of effector genes within such a pathogenicity island has been unknown up until the present invention.

## 10 SUMMARY OF THE INVENTION

In one aspect, the invention relates to a method for the identification of new  
15 virulence genes in bacterial strains containing a type III secretion system. In particular, the invention allows the identification of the effector virulence genes associated within a pathogenicity island containing the genes for the type III secretion system. Another aspect of the invention a method for the identification of pathogenic bacterial strains containing a type III secretion system. Another aspect of the invention relates to  
20 *Bordetella pertussis* BopN, Orf1, Orf2, Orf3, Orf4, Orf5, Orf6, Orf7, Orf8, Orf9, Orf10, Orf11, Orf12, Orf13, Orf14, Orf15 effector proteins, and the respective polynucleotide sequences encoding them.

Although the general concepts of type III secretion systems and pathogenicity  
25 islands have been reported, the problem of how simply and reliably to identify whether any given organism has such cell machinery has not been accomplished until now. Such a method is extremely useful to establish whether a given strain has a type III secretion system within a pathogenicity island, to characterise unknown virulence genes within the pathogenicity island, and to use in quick diagnostic methods for determining whether a  
30 cultured bacterial strain containing a type III secretion system is pathogenic.









The preferred method for identifying unknown pathogenicity islands comprising a type III secretion system is by:

- i) identifying two highly conserved regions of the target protein sequence (preferably of LcrD). Preferably, both regions should contain conserved amino acids which are encoded by the fewest number of codon possibilities e.g. Methionine (ATG being the only possibility) or Tryptophan (TGG being the only possibility). This minimises the number of permutations in both degenerate primer sets that are designed in the next stage of the process, thus ensuring a greater probability that each primer set will specifically anneal to the unknown *lcrD*-equivalent gene (thereby minimising background non-specific interactions). Most preferably, regions should also be chosen that are clearly distinguishable from the paralogue *flhA* flagellar genes, present in all flagellated bacterial strains.
- ii) designing a degenerate set of primers for both of the chosen regions such that a) the primers are at least 15 bases long, preferably 20-30 bases long, and still more preferably 21-23 bases long, b) they are degenerate at bases that can be more than one type of nucleotide whilst still encoding the same amino acid (due to the degeneracy of codon usage for amino acids), but no more degenerate than is required to cover all permutations for the amino acid region selected, and c) the primer set that encodes the more N-terminal region of the chosen protein should correspond to the coding strand of its corresponding double-stranded DNA sequence, and the set that encodes the more C-terminal region should correspond to the complementary strand of the corresponding double-stranded DNA sequence.
- iii) synthesising the degenerate primer sets of step ii) using conventional DNA synthesis methods well known in the art.
- iv) purifying the primer sets of step iii)
- v) adding both the primer sets and a sample containing nucleic acid from a bacterial strain (preferably a cell sample of the bacterial species itself) together in appropriate quantities and in an appropriate buffer in order to perform a polymerase chain reaction (PCR)

- vi) performing a PCR reaction in order to amplify the region of the gene between the two primers (conditions for performing the PCR reaction can be optimised using techniques well known in the art)
- vii) observing the reaction products on a gel (preferably an agarose gel) for an amplified product of the size expected; if no such product is present, the bacterial strain is unlikely to use a type III secretion system; if such a product is present, the bacterial strain is likely to have a type III secretion system, and is likely to be pathogenic.

The preferred method for confirming that the amplified product actually corresponds to a virulence gene is by carrying out steps i)-vii) above (where the target protein is LcrD) and then:

- viii) optionally separating the product of correct size from any background products of incorrect size by removing the correct band from the gel, purifying the product by conventional means, and amplifying the product once more with the two degenerate primer sets in another PCR reaction (under preferably more stringent PCR conditions) [this step is required should the product of step vii) not be pure enough for direct cloning]
- ix) inserting the DNA fragment by conventional means into a vector which is capable of being sequenced, and sequencing the fragment
- x) comparing the deduced amino acid sequence of ix) with that of known members of the LcrD/FlbF family of proteins to associate the amplified product as being part of either a virulence or a flagellar gene.

And optionally:

- xi) using the internal sequence of the fragment to design primers that are the exact sequence of, and specific to, the unknown *lcrD*-equivalent gene.
- xii) using the primers of xi) firstly to screen a genomic library of the organism for positive clones
- xiii) isolating the clones of xii), and sequence one or more of said clones

- xiv) scanning the sequence of one clone (and overlapping sequences of other clones) to search for an open reading frame which is approximately the same size as *lcrD* (approximately 2100bp), and encodes a protein homologous to LcrD
- xv) ascertaining whether the LcrD-equivalent protein is more homologous with the *flbF* (flagellar protein secretion) gene family or the *lcrD* (type III secretion system pathogenicity island) gene family.

The preferred method for characterising the whole pathogenicity island and defining unidentified virulence effector genes is by carrying out steps i)-xv) above (where the target protein is LcrD) and then:

- xvi) if the sequence is more homologous with the *lcrD* gene family, designing primers at either extreme of the gene sequence already ascertained, and scanning and sequencing the genomic library (using a standard chromosome walking strategy – where the insert boundaries of an original clone serves as a probe for screening and cloning adjacent regions) to sequence eventually the whole of the pathogenicity island (both boundaries of which will be defined by the presence of either direct or inverted repeats, or insertion sequences, or the presence of house-keeping genes)
- xvii) defining unidentified virulence effector genes within the sequenced pathogenicity island
- xviii) cloning, expressing and characterising the virulence genes of xvii) which encode virulence effector proteins of the organism

### Definitions

“Bordetella pathogenicity proteins” refers generally to polypeptides having the amino acid sequence encoded by the genes defined in tables 2 and 3, or an allelic variant thereof. These proteins are: BcrD, BcrH, BscC, BscD, BscE, BscF, BscI, BscJ, BscK, BscL, BscN, BscO, BscP, BscQ, BscR, BscS, BscT, BscU, BscV, BrpL, BopN, Orf1, Orf2, Orf3, Orf4, Orf5, Orf6, Orf7, Orf8, Orf9, Orf10, Orf11, Orf12, Orf13, Orf14, Orf15.

“Bordetella pathogenicity genes” refers to polynucleotides having the nucleotide sequence defined in tables 2 and 3, or allelic variants thereof and/or their complements. These genes are: *bcrD*, *bcrH*, *bscC*, *bscD*, *bscE*, *bscF*, *bscI*, *bscJ*, *bscK*, *bscL*, *bscN*,  
5 *bscO*, *bscP*, *bscQ*, *bscR*, *bscS*, *bscT*, *bscU*, *bscV*, *brpL*, *bopN*, *orf1*, *orf2*, *orf3*, *orf4*, *orf5*,  
*orf6*, *orf7*, *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*.

“Polypeptide” refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide  
10 isosteres. “Polypeptide” refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. “Polypeptides” include amino acid sequences modified either by natural processes, such  
15 as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-  
20 chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with  
25 or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent  
30 attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation,

proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and  
5 Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational  
10 Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA,  
15 DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA  
20 and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms  
25 of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that  
30 differs from a reference polynucleotide or polypeptide respectively, but retains essential

properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions (preferably conservative), additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis. Variants should retain one or more of the biological activities of the reference polypeptide. For instance, they should have similar (preferably the same) antigenic or immunogenic activities as the reference polypeptide. Antigenicity can be tested using standard immunoblot experiments, preferably using polyclonal sera against the reference polypeptide. The immunogenicity can best be tested by measuring antibody responses (using polyclonal sera generated against the variant polypeptide) against purified reference polypeptide in a standard ELISA test. Preferably, a variant would retain all of the above biological activities.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New

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York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heijne, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403). Most preferably, the program used to determine identity levels was the GCG 9 package, as was used in the Examples below.

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include on average up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal

positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

## 5 Polypeptides of the invention

In one aspect, the present invention relates to *Bordetella* pathogenicity proteins (or polypeptides). The *Bordetella* pathogenicity polypeptides include the polypeptides encoded by the genes defined in tables 2 and 3; as well as polypeptides comprising the amino acid sequence encoded by the genes defined in tables 2 and 3; and polypeptides  
10 comprising the amino acid sequence which have at least 75% identity to that encoded by the genes defined in tables 2 and 3 over their entire length, and preferably at least 80% identity, and more preferably at least 90% identity. Those with 95-99% identity are highly preferred.

15 The *Bordetella* pathogenicity polypeptides (or fragments thereof) may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It may be advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues or Maltose Binding Protein (MBP), or an additional sequence  
20 for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

Fragments of the *Bordetella* pathogenicity polypeptides are also included in the  
25 invention. A fragment is a polypeptide having an amino acid sequence that is the same as part, but not all, of the amino acid sequence of the aforementioned *Bordetella* pathogenicity polypeptides. As with *Bordetella* pathogenicity polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide  
30 fragments of the invention, include, for example, fragments from about amino acid number



1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of Bordetella pathogenicity polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes. The fragments should comprise at least 7 consecutive amino acids from the sequences e.g. 8, 10, 12, 14, 18, 20 or more depending on the particular sequence). Preferably the fragments comprise an epitope from the sequence.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of Bordetella pathogenicity polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus and/or transmembrane region or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate Bordetella pathogenicity protein activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity (for instance antigenic or immunogenic) of the Bordetella pathogenicity protein, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic

residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination. Most preferred variants are naturally occurring allelic variants of Bordetella pathogenicity polypeptide present in strains of *Bordetella pertussis*.

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The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

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The Bordetella pathogenicity polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

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It is most preferred that a polypeptide of the invention is derived from *Bordetella pertussis*, however, it may preferably be obtained from other organisms of the same taxonomic genus. A polypeptide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order, such as *Bordetella parapertussis* or *Bordetella bronchiseptica*.

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A further aspect of the invention is substantially purified Bordetella pathogenicity polypeptides of the invention. "substantially purified" when used in reference to a protein or peptide means that the molecule has been largely, but not necessarily wholly, separated and purified from other cellular and non-cellular components. Typically a protein is substantially pure when it is at least about 60 % by weight free from other

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naturally occurring organic molecules. Preferably the purity is at least about 75 %, more preferably at least about 90% , and most preferably at least about 99% by weight pure.

## Polynucleotides of the invention

Another aspect of the invention relates to Bordetella pathogenicity polynucleotides. Bordetella pathogenicity polynucleotides include isolated polynucleotides which encode the Bordetella pathogenicity polypeptides and fragments respectively, and polynucleotides closely related thereto or variants thereof. More specifically, Bordetella pathogenicity polynucleotides of the invention include a polynucleotide comprising the nucleotide sequence of genes defined in table 2 or 3, encoding a Bordetella pathogenicity polypeptide. Bordetella pathogenicity polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 75% identity over its entire length to a nucleotide sequence encoding the Bordetella pathogenicity polypeptide encoded by the genes defined in tables 2 and 3, and a polynucleotide comprising a nucleotide sequence that is at least 75% identical to that of the genes defined in tables 2 and 3. In this regard, polynucleotides at least 80% identical are particularly preferred, and those with at least 90% are especially preferred. Furthermore, those with at least 95% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under Bordetella pathogenicity polynucleotides is a nucleotide sequence which has sufficient identity to a nucleotide sequence of a gene defined in tables 2 and 3 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such Bordetella pathogenicity polynucleotides.

Using the information provided herein, such as specific *Bordetella* pathogenicity gene and polypeptide sequences, a polynucleotide of the invention encoding a *Bordetella* pathogenicity polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Bordetella pertussis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, typically a

library of clones of chromosomal DNA of *Bordetella pertussis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Bordetella pertussis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising a sequence defined in table 2 or 3 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence defined in tables 2 and 3 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence defined in table 2 or 3 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers are described elsewhere herein.



which several, 10-25, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Most preferred variant polynucleotides are those naturally occurring *Bordetella pertussis* sequences that encode allelic variants of the *Bordetella* pathogenicity proteins in *Bordetella* strains, preferably *B. pertussis*.

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The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

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Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence of any gene defined in tables 2 and 3 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding *Bordetella* pathogenicity polypeptides respectively and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than *Bordetella pertussis*) that have a high sequence similarity to the *Bordetella* pathogenicity genes. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides. In one embodiment, to obtain a polynucleotide encoding *Bordetella* pathogenicity polypeptide, including homologs and orthologs from species other than *Bordetella pertussis*, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having a nucleotide sequence contained in one of the gene sequences defined by tables 2 and 3, or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Thus in another aspect, *Bordetella* pathogenicity polynucleotides of the present invention further

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include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having a nucleotide sequence contained in one of the genes defined by table 2 and 3, or a fragment thereof. Also included with *Bordetella* pathogenicity polypeptides are polypeptides comprising amino acid sequences encoded by nucleotide sequences obtained by the above hybridization conditions. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

A coding region of a *Bordetella* pathogenicity gene may be isolated by screening using a DNA sequence defined in table 2 or 3 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA* 85: 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5'

in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

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The polynucleotides of the invention that are oligonucleotides derived from a sequence defined in table 2 or 3 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

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The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

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### Diagnostic Assays

This invention also relates to the use of Bordetella pathogenicity polypeptides, or Bordetella pathogenicity polynucleotides, for use as diagnostic reagents. Detection of Bordetella pathogenicity polypeptides will provide a diagnostic tool that can add to or define a diagnosis of *B. pertussis* disease, among others.

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Materials for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy.

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Thus in another aspect, the present invention relates to a diagnostic kit for a disease or susceptibility to a disease, particularly *B. pertussis* disease, which comprises:

- (a) a Bordetella pathogenicity polynucleotide, preferably the nucleotide sequence of one of the gene sequences defined by tables 2 and 3, or a fragment thereof;
- (b) a nucleotide sequence complementary to that of (a);

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- (c) a *Bordetella* pathogenicity polypeptide, preferably the polypeptide encoded by one of the gene sequences defined in tables 2 and 3, or a fragment thereof;
- (d) an antibody to a *Bordetella* pathogenicity polypeptide, preferably to the polypeptide encoded by one of the gene sequences defined in tables 2 and 3; or
- 5 (e) a phage displaying an antibody to a *Bordetella* pathogenicity polypeptide, preferably to the polypeptide encoded by one of the gene sequences defined in tables 2 and 3.

It will be appreciated that in any such kit, (a), (b), (c), (d) or (e) may comprise a substantial component.

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Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides from any of these sources, particularly DNA or RNA, may be used directly for detection or may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected polynucleotide of the organism. Deletions and insertions can be detected by a change in size of the amplified product in comparison to a genotype of a reference sequence selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled *Bordetella* pathogenicity polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished from imperfectly or more significantly mismatched duplexes by DNase or RNase digestion, for DNA or RNA respectively, or by detecting differences in melting temperatures or renaturation kinetics. Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by direct DNA or RNA sequencing. See, for example, Myers *et al.*, *Science*, 230: 1242 (1985). Sequence changes at specific locations also may be

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revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method. See, for example, Cotton *et al.*, *Proc. Natl. Acad. Sci., USA*, 85: 4397-4401 (1985).

5 This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention, which is associated with a disease or pathogenicity will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results from under-expression, over-  
10 expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such as those described elsewhere herein.

The invention further provides a process for diagnosing disease, preferably bacterial  
15 (particularly *Bordetella*) infections, more preferably infections caused by *Bordetella pertussis*, comprising determining from a sample derived from an individual, such as a bodily material, an increased level of expression of polynucleotide having a sequence defined in table 2 or 3. Increased or decreased expression of a polynucleotide can be measured using any one of the methods well known in the art for the quantitation of  
20 polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting, spectrometry and other hybridization methods.

### **Vectors, Host Cells, Expression Systems**

The invention also relates to vectors that comprise a polynucleotide or  
25 polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems that comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci, *E. coli*, streptomyces, cyanobacteria, *Bacillus subtilis*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Neisseria meningitidis*; fungal cells, such as cells of a yeast, *Kluyveromyces*, *Saccharomyces*, a basidiomycete, *Candida albicans* and *Aspergillus*; insect cells such as cells of *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia

viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses, retroviruses, and alphaviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender  
5 expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

10 In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be  
15 heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose  
20 chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

25 The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses.

30 Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia,

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fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelan Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), Listeria, Salmonella, Shigella, Neisseria, BCG. These viruses and bacteria can be virulent, or attenuated in  
5 various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

### Antibodies

According to a further aspect, the invention provides antibodies which bind  
10 specifically to the polypeptides of the invention. These may be polyclonal or monoclonal and may be produced by any suitable means well known to a skilled person in the art.

Typically, a mouse or rat is immunised with a protein (preferably adjuvanted with Freund's complete adjuvant) and injected (doses of 50-200 µg/injection is typically  
15 sufficient). Polyclonal antibodies can be isolated by bleeding the animal to extract serum. Alternatively, monoclonal antibodies can be generated by removing the spleen (or large lymph nodes) and dissociating it into single cells (Kohler and Milstein, (1975) Nature, 256:495-497). These are then induced to fuse with myeloma cells to form hybridoma, and are cultured in a selective medium (eg hypoxanthine, aminopterin, thymidine  
20 merium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected monoclonal-secreting hybridomas are then cultured either in vitro (eg in tissue culture bottles or hollow fiber  
reactors), or in vivo (as Ascites in mice).

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Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this invention. Also, transgenic mice, or other organisms or animals, such as other mammals, may be used to express humanized antibodies immunospecific to the polypeptides or  
30 polynucleotides of the invention.

Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards a polypeptide of the invention either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-Bordetella pathogenicity polypeptide or from naive libraries (McCafferty, *et al.*, (1990), Nature 348, 552-554; Marks, *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson *et al.*, (1991) *Nature* 352: 628).

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention to purify the polypeptides or polynucleotides by, for example, affinity chromatography.

Antibodies against a Bordetella pathogenicity polypeptide or polynucleotide may be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants form a particular aspect of this invention.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized," where the complementarity determining region or regions of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones *et al.* (1986), *Nature* 321, 522-525 or Tempest *et al.*, (1991) *Biotechnology* 9, 266-273.

### Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with Bordetella pathogenicity polypeptide or epitope-bearing fragments, analogs, outer-

membrane vesicles or cells (attenuated or otherwise) adequate to produce antibody and/or T cell immune response to protect said animal from Bordetella (particularly *B. pertussis*) disease, among others. Such agents may be used alone, or conjugated to another molecule which improves its immunological potency. In particular the invention relates to the use of Bordetella pathogenicity polypeptides encoded by the genes defined in table 3 – the effector proteins. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering Bordetella pathogenicity polypeptide via a vector directing expression of Bordetella pathogenicity polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

A further aspect of the invention relates to an immunological composition or vaccine formulation which, when introduced into a mammalian host, induces an immunological response in that mammal to a Bordetella pathogenicity polypeptide (particularly one encoded by a gene defined in table 3) wherein the composition comprises a Bordetella pathogenicity gene, or Bordetella pathogenicity polypeptide or epitope-bearing fragments, analogs, outer-membrane vesicles or cells (attenuated or otherwise). The vaccine formulation may further comprise a suitable carrier. The Bordetella pathogenicity polypeptide vaccine composition is preferably administered orally or parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems

known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

The vaccine formulations of the invention may also comprise other *Bordetella* antigens known to be suitable vaccinal agents, for instance: pertussis toxoid, pertactin, agglutinogens 1 and 2, FHA (filamentous haemagglutinin), and adenylate cyclase / haemolysin (AC/HLY), or immunogenic fragments thereof (Locht *et al.*, NAR (1986) 14:3251-3261; Relman *et al.*, PNAS USA (1989) 86:2637-2641; Roberts *et al.*, Mol. Microbiol. (1991) 5:1393-1404; Mooi *et al.*, Microb. Pathog. (1992) 12:127-135; Hewlett and Gordon, In *Pathogenesis and Immunity in Pertussis* (1988), New York, Wiley & Sons, pp. 193-209.

Yet another aspect of the invention relates to an immunological/vaccine formulation which comprises the polynucleotide of the invention. Such techniques are known in the art, see for example Wolff *et al.*, *Science*, (1990) 247: 1465-8.

Vaccine compositions can comprise polypeptides, antibodies, or polynucleotides of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition (in this case *Bordetella*, particularly *B. pertussis*, disease), or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention.



## EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail.

5 The examples illustrate, but do not limit the invention.

Example 1: A type III secretion system is present in a pathogenicity island in *Bordetella pertussis*.

The presence of a *lcrD* homologous gene in the *Bordetella pertussis* genome was investigated by polymerase chain reaction (PCR). The primers used (oligos 95080 and 10 95081 shown in Table 1) were degenerate oligonucleotides corresponding to highly conserved regions of the amino acids sequences of the LcrD/FlbF family of proteins. These primers were also designed to favour the amplification of virulence genes instead of their paralogue *flhA* or *flbF* flagellar genes, present in flagellated bacterial strains. The 15 presence of the 3' triplet CAT in oligonucleotide 95081 is a determinant – indeed when multiple sequence analysis is done using known homologous sequences (database searching was done with either the FASTA and TFASTA programs of the GCG9 package, or with BLASTN, BLASTP and BLASTX programs, and alignments were carried out with the PILEUP program from the GCG9 package) it could be seen that the 20 CAT triplet codes for a methionine which is exclusively present in virulence sequences while absent in the flagellar ones.

When analysed on agarose gel, the PCR product appeared as a heterogeneous mix of fragments, one of which was presenting the expected size (around 150 bp). A second 25 round of amplification using the approximately 150 bp DNA as template yielded a single amplicon which was cloned in pCRII (obtained from Invitrogen) for further characterisation. It appeared as a 152 bp fragment whose nucleotide sequence (Fig. 1), although similar to all *lcrD/flbF* homologous genes, shares a higher level of identity with the virulence (*lcrD*-like) genes.

30

Table 1.

| oligonucleotides | sequence <sup>1</sup>          | features                   | <i>lcrD</i> corresponding codons <sup>2</sup> |
|------------------|--------------------------------|----------------------------|---|
| 95080            | GSH ATG CCW GGH AAR CAR ATG    | direct, degenerate         | 150 to 156                                    |
| 95081            | GC RTC DCC YTT DAC RAA YTT CAT | complement, degenerate     | 193 to 200                                    |
| 95363            | CC ATC GAC GCG GAC TTG CGC G   | direct, non-degenerate     | 157 to 164                                    |
| 95364            | CGC GCC GTC CAT GGC GCC ATA    | complement, non-degenerate | 186 to 192                                    |
| 96110            | C CGA CGC CGA CGC CGT ACG GTC  | direct, non-degenerate     | 172 to 179                                    |

<sup>1</sup> The letter code for nucleotide ambiguity proposed by IUB (Nomenclature Committee, 1985, Eur. J. Biochem., **150**: 1-5) was used.

<sup>2</sup> The DNA sequence of the *lcrD* gene from *Yersinia enterocolitica* used for this work was published by Plano *et al.* (1991).

To ensure that the cloned fragment was actually a *B. pertussis* sequence PCR was performed under stringent conditions with serial 10-fold dilutions of DNA from *B. pertussis*. The optimisation of stringent PCR conditions require a perfect match between template and primers. It was likely, however, that due to the degeneration of the original primers, the 152 bp sequence initially obtained had, at its boundaries, a few base pair differences with the actual *B. pertussis lcrD*-like (hereafter called *bcrD*) sequence. A nested PCR approach using internal primers (oligos 95363 and 95364 Table 1) was therefore preferred, as primers known to be the correct *B. pertussis* sequence are used. A dose-response-relationship was observed between the 10-fold dilutions of *B. pertussis* template DNA and the product of the nested PCR, suggesting that the 152 bp amplicon actually originates from the *Bordetella* genome.

Comparison of the 152 bp sequence with *lcrD/flbF* genes allowed us to define a specific DNA stretch (oligo 96110 in Table 1) which was used as a probe for screening a

genomic library of *B. pertussis* constructed in the plasmid vector pBR327 (Delisse-Gathoye *et al*, 1990, *Infect-Immun.* 58: 2895-905). Several positive clones were isolated and restriction analysis of their resident plasmids showed that they harboured overlapping inserts. The entire nucleotide sequence of one insert was determined,  
5 revealing a large open reading frame (ORF). This 2100 bp ORF encoded a 75 kDa polypeptide which is 59 % and 47 % identical to the yersinial proteins LcrD and FlhA respectively. Multiple amino acids comparisons of all known members of the LcrD/FlhF family of proteins, including the *B. pertussis* BcrD deduced amino acid sequence, showed that this sequence clearly ranked within the virulence associated determinants  
10 (Fig. 2). These data strongly suggest that *B. pertussis* possesses a type III export system, involved in the secretion of virulence effectors.

The *B. pertussis* *lcrD*-like nucleotide sequence (*bcrD*) has been submitted to EMBL and assigned the accession number Y13383.

This general technique has been useful for determining the presence/absence of a type III secretion system in other bacterial strains. The human pathogens *Borrelia burgdorferi* and *Helicobacter pylori* were intensively screened for such a system using this technique. No evidence for a type III secretion system could be found. The  
20 subsequent publication of the genome sequences of these microorganisms has confirmed the absence of similar systems in these species. In contrast, the method allowed the amplification of a DNA fragment from the phytopathogen *Pseudomonas corrugata*, which clearly ranks among the virulence sequences. This technique could be applied to any Gram negative pathogen of medical or agronomic importance such as *Neisseria* spp,  
25 *Moraxella catharalis*, *Vibrio cholerae*, any Enterobacteriaceae, *Pseudomonas* spp, *Haemophilus influenzae*, *Brucella* spp, *Francisella tularensis*, *Pasteurella* spp, *Legionella pneumophila*. Even in strains that have been fully sequenced, this technique can be used as a simple method for checking alternate types or strains of the same species. For instance, some types of pathogenic *Escherichia coli* harbour a type III  
30 secretion system whereas others do not.



**Table 2**

| names   | Coding sequence<br>from/to (with<br>reference to Fig. 5) | Coding<br>DNA strand | SEQ ID<br>NO: | Homologous genes (from<br><i>Yersinia</i> , unless<br>otherwise specified) |
|---|--|----------------------|---------------|--|
| Class I genes, i.e. genes coding for determinants involved in the secretory apparatus<br>and their regulation |  |                      |               |  |
| <i>bcrD</i>   | 8656/10755   | complement           | 1             | <i>LcrD</i>  |
| <i>bcrH</i>   | 14097/14582  | direct               | 3             | <i>lcrH</i> (= <i>syncD</i> )  |
| <i>bscC</i>   | 26955/28757  | direct               | 5             | <i>YscC</i>  |
| <i>bscD</i>   | 7379/8659  | complement           | 7             | <i>YscD</i>  |
| <i>bscE</i>   | 7039/7338  | complement           | 9             | None   |
| <i>bscF</i>   | 6783/7049  | complement           | 11            | <i>YscF</i>  |
| <i>bscI</i>   | 17892/18218  | direct               | 13            | <i>YscI</i>  |
| <i>bscJ</i>   | 18215/19039  | direct               | 15            | <i>YscJ</i>  |
| <i>bscK</i>   | 19032/19694  | direct               | 17            | None   |
| <i>bscL</i>   | 19664/20302  | direct               | 19            | <i>YscL</i>  |
| <i>bscN</i>   | 20307/21641  | direct               | 21            | <i>YscN</i>  |
| <i>bscO</i>   | 21641/22150  | direct               | 23            | <i>YscO</i>  |
| <i>bscP</i>   | 22147/22695  | direct               | 25            | None   |
| <i>bscQ</i>   | 22692/23771  | direct               | 27            | <i>YscQ</i>  |
| <i>bscR</i>   | 23768/24439  | direct               | 29            | <i>YscR</i>  |
| <i>bscS</i>   | 24445/24711  | direct               | 31            | <i>YscS</i>  |
| <i>bscT</i>   | 24723/25523  | direct               | 33            | <i>YscT</i>  |
| <i>bscU</i>   | 25520/26569  | direct               | 35            | <i>YscU</i>  |
| <i>bscV</i>   | 26566/26964  | direct               | 37            | None   |
| <i>brpL</i>   | 28778/29380  | complement           | 39            | <i>hrpL</i><br>( <i>Pseudomonas syringae</i> )                             |

**Table 3**

| Names   | Coding sequence from/to (with reference to Fig. 5) | Coding DNA strand | SEQ ID NO: | Homologous genes (from <i>Yersinia</i> , unless otherwise specified) |
|---|--|-------------------|------------|--|
| Class II ORFs which putatively code for effector proteins |  |                   |            |  |
| <i>bopN</i>   | 11906/13003  | complement        | 41         | <i>YopN</i> (= <i>lcrE</i> )   |
| <i>orf1</i>   | 6160/6747  | direct            | 43         | None   |
| <i>orf2</i>   | 10752/11120  | complement        | 45         | None   |
| <i>orf3</i>   | 11117/11527  | complement        | 47         | None   |
| <i>orf4</i>   | 11532/11909  | complement        | 49         | None   |
| <i>orf5</i>   | 13002/13784  | direct            | 51         | None   |
| <i>orf6</i>   | 13806/14081  | direct            | 53         | None   |
| <i>orf7</i>   | 14630/15571  | direct            | 55         | None   |
| <i>orf8</i>   | 15601/16803  | direct            | 57         | None   |
| <i>orf9</i>   | 16827/17288  | direct            | 59         | <i>BcrH</i>  |
| <i>orf10</i>  | 17293/17814  | direct            | 61         | <i>pcr4</i><br>( <i>Pseudomonas aeruginosa</i> )                     |
| <i>orf11</i>  | 29412/29591  | complement        | 63         | None   |
| <i>orf12</i>  | 29555/30529  | complement        | 65         | None   |
| <i>orf13</i>  | 30631/31776  | direct            | 67         | None   |
| <i>orf14</i>  | 31773/33005  | complement        | 69         | None   |
| <i>orf15</i>  | 32370/33014  | direct            | 71         | None   |

**Table 4**

| No name specified                           | Coding sequence from/to (with reference to Fig. 5) | Coding DNA strand | SEQ ID NO: | Homologous sequences                       |
|---|--|-------------------|------------|--|
| Insertion Sequences and house keeping genes |  |                   |            |  |
|   | 711/2024   | direct            | 73         | uracil permease genes of numerous bacteria |
|   | 2055/3590  | complement        | 75         | Chemoreceptor genes of numerous bacteria   |
|   | 4220/4696  | direct            | 77         | <i>greA</i> ( <i>Escherichia coli</i> )    |
|   | 4998/5948  | complement        | 79         | transposase genes of numerous bacteria     |
|   | 33002/34852  | complement        | 81         | ICFG gene ( <i>Synechocystis</i> sp)       |

Next to the *bcrD* gene, there is an open reading frame (ORF) whose deduced amino acid sequence shares significant similarities with the YscU protein of *Yersinia* spp (39% identity and 51% similarity) and other known YscU homologs (Fig. 4). YscU, like LcrD, is a component of the *Yersinia* type III secretion machinery involved in the virulence mechanisms of the bacteria. *B. pertussis* therefore possesses a classical type III secretion system which is most probably involved in pathogenicity. This latter point can be investigated through phenotypic analyses of mutants (see below).

The total length of the Pai is approximately 30 to 40 kb. The DNA sequence of the whole region is presented in Figure 5, and is referred to in tables 2, 3, and 4. Restriction analysis on pulsed-field gel electrophoresis allowed the type III locus to be mapped at coordinate position 1,590 kb on the Tohama I strain chromosome.

No homologies could be found between the *B. pertussis* Class II Pai DNA sequences and the sequences reported in the GenEMBL databases (except for those stated in table 3). The expressed products of these unknown genes within the Pai





with *lacZ*, used as a reporter gene. The resulting construct was named pAF245. Similarly, primers were designed for placing *lacZ* downstream of a 849 bp fragment that encompassed upstream *bscN* sequences including its 3 first codons. pAF246 was obtained by cloning this fragment in pNM480.

5

#### *Transformations and allelic exchanges*

*B. pertussis* cells, from a freshly saturated culture in 10 ml of SS medium, were washed and resuspended in 100µl of a cold 10% (v/v) glycerol solution. Up to 10 µg of supercoiled purified DNA in a maximum of 20 µl of water were added to 100 µl of the bacterial suspension. Cells and DNA were transferred to a prechilled 0.2 cm electroporation cuvette (Bio-Rad) and placed in a Gene Pulser apparatus (Bio-Rad). Pulses were achieved with settings of 25 µF, 2.5 kV, and 600 Ω, giving a time constant ranging from 11 to 14 ms.

10

15

After their initial isolation on BG plus gentamycin, pAF214 and pAF248 transformants that undergone a second recombination step were selected on streptomycin as described (Stibitz, *supra*). The null *bcrD* mutants were finally distinguished from revertants by their acquired resistance to kanamycin. The proper integration of the *aphA-3* was assessed by southern blot analysis. In contrast, introduction of pAF245 and pAF246 only required a single crossover selected on BG plus ampicillin. This recombination step led to the placement of the *lacZ* coding sequence under the control of the signals governing the transcription of *bcrD* and *bscN* respectively.

20

#### *Mice model*

After a two days growing on BG agar plates, wild type and mutant bacteria were recovered and resuspended in PBS at a concentration of  $10^8$  PFU ml<sup>-1</sup>. 25 µl of the suspension were injected in each nostril of pentobarbital anaesthetized mice. Lungs colonization was assayed after 4 h, 3, 7, 14, 26, 39 and 45 days by treating both lungs of each mouse in an Ultraturax grinder and titrating the resuspended bacteria on BG agar plates.

25

30

### *$\beta$ -galactosidase assay*

0.5 ml of bacterial suspensions coming from liquid cultures grown to log phase (OD = 0.2), were assayed as described previously (Miller, (1972) "Experiments in molecular genetics." Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). We used the chromogenic substrate *o*-nitrophenyl- $\beta$ -D-galactoside (ONPG) of Sigma.

*Transcription of both bcrD and bscN transcripts appear controlled by the bvg locus*

Most of the *Bordetella* virulence functions are controlled by the *bvg* locus. The Bvg<sup>+</sup> phase is characterized by the expression of virulence factors and is necessary for colonization of animal models. In contrast, the bacteria are avirulent in Bvg<sup>-</sup> phase which can be induced by nicotinic acid or MgSO<sub>4</sub>. We investigated the level of expression of two genes that belonged to distinct unit of transcription, i.e. *bcrD* and *bscN*, by using transcriptional fusions of *lacZ* into these genes. To this end, we isolated the mutants NIVh86 and NIVh87, which integrated pAF245 and pAF246 respectively. In the former mutant, a single recombination step led to the setting *lacZ* in place of the *bcrD* coding sequence, whereas in the latter, *lacZ* replaced *bscN*. The level of expression of both *bcrD* and *bscN* transcripts was assessed either in Bvg<sup>+</sup> or in Bvg<sup>-</sup> phases. Both *B. pertussis* genes were weakly expressed *in vitro*. Additionally, however, these levels of expression appeared to be clearly modulated by the Bvg system. Indeed, whereas  $\beta$ -galactosidase could be assayed in Bvg<sup>-</sup> conditions, no enzyme activity was detected in Bvg<sup>+</sup> phase (table 5).

**Table 5.**  $\beta$ -galactosidase activity, in Miller units (Miller, *supra*), when *lacZ* is placed under the control of that direct the expression of *bcrD* or *bscN*.

| phase<br>transcript | Bvg <sup>+</sup> | Bvg <sup>-</sup> |
|---------------------|------------------|------------------|
| <i>bcrD</i>         | 3.54             | 0.02             |
| <i>bscN</i>         | 1.65             | 0.04             |

5

Example 4: Recombinant expression of effector protein vaccine candidates

In the discovered sequence, seven ORFs (*orf2* to *-8*) particularly fulfil certain criteria that make them good candidates as effector proteins and vaccine candidates.

10 First, they appear surrounded by typical type III secretion (class I) genes, and therefore incontestably belong to the type III secretion locus. Furthermore, they don't display significant similarities with genes present in related type III systems from other organisms, and are therefore likely to be effector proteins specific for *Bordetella*. In addition to these ORFs *bopN*, *orf9* and *orf10* are also of particular interest as vaccine

15 candidates. Despite the fact that these sequences do not fulfil the second criterium above (they have some similarity to *popN*, *pcrH* and *pcr4* of *Pseudomonas aeruginosa*), these products may also be exported by the specialized translocon. For these reasons, ten ORFs, i.e. *orf2* to *-10* and *bopN*, were selected for further analysis. To this end, ten pairs of primers (table 6) were designed for amplifying their corresponding ORF. The

20 amplified ORFs were then cloned in the pCR-TOPO<sup>®</sup> T/A cloning system (Invitrogen) and their sequences were checked for errors putatively induced by the Taq DNA polymerase. Correct inserts were retrieved by *EcoRI* and *BamHI* (or *BglII* - see table 6) cutting and transferred into the pMAL<sup>®</sup> vectors (New England Biolabs; Maina *et al.*, Gene (1988) 74:365-373), opened by *EcoRI* and *BamHI* restriction. In these vectors,

25 expression of the cloned inserts yields recombinant proteins fused to the maltose binding

protein (MBP) of *E. coli*. The MBP domain of the fusion protein provides a means for both detecting the expressed product and purifying it by affinity chromatography.

Four ORFs, namely *orf2*, *-4* and *-10* on the one hand, and *orf6* on the other, have been cloned into pMAL-c2E<sup>®</sup> and pMAL-p2E<sup>®</sup> respectively. Transformed bacteria, grown in 300 ml of culture medium, were induced with IPTG (300 µM) and lysed in a French pressure cell. Insoluble material was pelleted by ultracentrifugation and discarded whereas the resulting supernatant was applied to an amylose resin. Fusion proteins that specifically bind to the amylose through their MBP domain, were further eluted by application of maltose 10 mM. This method allowed us to recover from 10 to 50 mg of each fusion protein (Fig. 6). The expressed *Bordetella* products may be separated from the MBP by utilising the enterokinase cleavage site between the *Bordetella* polypeptide and the MBP. The other ORFs should be expressible using a similar approach.

The secreted proteins will be analysed using standard techniques to confirm their functional and immunological properties. First, the immunogenicity of the secreted proteins will be assessed by investigating the presence of antibodies directed against these proteins in the serum of infected patients. In addition, their putative recognition as protective antigens will be based on challenge experiments, realized in a mouse model. Second, the biological properties of the effector proteins will be assessed by analysing their catalytic activities. For instance, it is expected that one of the secreted proteins would display a tyrosine phosphatase activity. Finally, the function of the effector proteins will be investigated by microinjecting the proteins into the cytoplasm of eukaryotic cells. This will allow us to display putative activities of inhibition of actin polymerisation, cytotoxicity or induction of apoptosis, i.e. those types of activities that have been assigned to effector proteins secreted by type III secretion systems discovered in other species.



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- 25

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Alain Fauconnier  
Edmond Godfroid

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|     | Val | Phe | Met | Met | Ile | Leu | Pro | Leu | Pro | Thr | Thr | Leu | Val | Asp | Val | Leu |
|     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
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|     | Ile | Gly | Ala | Asn | Met | Thr | Leu | Ser | Ala | Val | Leu | Leu | Met | Val | Ala | Met |
|     |     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |
| 45  | tac | ctg | cct | tcg | ccc | ctg | gcg | ttt | tcc | tcg | ttc | cct | tcg | gtc | ctg | ctg |
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|     | Leu | Thr | Ile | Val | Gln | Phe | Val | Val | Ile | Thr | Lys | Gly | Ala | Glu | Arg | Val |
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| 40 | gca tat gta ttg aaa aaa tat tca gcc gat ttc atc ggc atc cag gaa<br>Ala Tyr Val Leu Lys Lys Tyr Ser Ala Asp Phe Ile Gly Ile Gln Glu<br>500 505 510     | 1536 |
| 45 | acc cgg ttt ctg ctt tcc gcc atg gaa gaa cga ttt ccc gat ctg gtc<br>Thr Arg Phe Leu Leu Ser Ala Met Glu Glu Arg Phe Pro Asp Leu Val<br>515 520 525     | 1584 |
| 50 | aag gag tgc ctg cgc gtc atg ccg gtg cag aag att gcc gaa atc ctg<br>Lys Glu Cys Leu Arg Val Met Pro Val Gln Lys Ile Ala Glu Ile Leu<br>530 535 540     | 1632 |
| 55 | cag cgc ctt gtt tcc gaa gaa gtg tcc ata cgc aac ctg cgc gcc gtc<br>Gln Arg Leu Val Ser Glu Glu Val Ser Ile Arg Asn Leu Arg Ala Val<br>545 550 555 560 | 1680 |
| 60 | ctg gaa gcg ctg gtc gaa tgg ggc cag aag gaa aag gat acc gtc ctg<br>Leu Glu Ala Leu Val Glu Trp Gly Gln Lys Glu Lys Asp Thr Val Leu<br>565 570 575     | 1728 |
| 65 | ctt acg gag tat gtc cga atc gca ctc aag cgc tat atc agc cac aag<br>Leu Thr Glu Tyr Val Arg Ile Ala Leu Lys Arg Tyr Ile Ser His Lys<br>580 585 590     | 1776 |
| 70 | tac acc agc ggc cac aat atc ctg ccc gcc tac ctg ctg gcc ccc aag<br>Tyr Thr Ser Gly His Asn Ile Leu Pro Ala Tyr Leu Leu Ala Pro Lys<br>595 600 605     | 1824 |
| 75 | gtc gag gaa acc gtg cgc gcc gcc atc cgg cag acc gcc gcc ggc agt<br>Val Glu Glu Thr Val Arg Ala Ala Ile Arg Gln Thr Ala Ala Gly Ser<br>610 615 620     | 1872 |

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|    |  |      |
|----|--|------|
| 5  | tat ctc gcc ctc gat ccg gac acg aca cgc cga ctg gtc gag cac atc<br>Tyr Leu Ala Leu Asp Pro Asp Thr Thr Arg Arg Leu Val Glu His Ile<br>625 630 635 640  | 1920 |
| 10 | cgt caa tgt gtc ggc gat ctg gcc gcc ggc gcg agc cgt ccc gtc ttg<br>Arg Gln Cys Val Gly Asp Leu Ala Ala Gly Ala Ser Arg Pro Val Leu<br>645 650 655  | 1968 |
| 15 | ctg acg tcg atg gac atc cgg cgc tac acg cgc aag atg ata gaa gcc<br>Leu Thr Ser Met Asp Ile Arg Arg Tyr Thr Arg Lys Met Ile Glu Ala<br>660 665 670  | 2016 |
| 20 | gat ctc tac gcc ctg ccg gtg ctg tcc tac cag gaa ctg acg ccg gag<br>Asp Leu Tyr Ala Leu Pro Val Leu Ser Tyr Gln Glu Leu Thr Pro Glu<br>675 680 685  | 2064 |
| 25 | atc aat gta cag ccc ctg ggc agg gtg gat cta tga<br>Ile Asn Val Gln Pro Leu Gly Arg Val Asp Leu *   | 2100 |
| 30 | <210> 2<br><211> 699<br><212> PRT<br><213> Bordetella pertussis<br><br><400> 2<br>Met Thr Ser Lys Lys Ser Ile Arg Arg Leu Gln Arg Ala Val Ala Leu<br>1 5 10 15<br>Ala Thr Ser Arg Asn Asp Ile Val Leu Ala Val Leu Ile Val Ala Ile<br>20 25 30<br>Val Phe Met Met Ile Leu Pro Leu Pro Thr Thr Leu Val Asp Val Leu<br>35 40 45<br>Ile Gly Ala Asn Met Thr Leu Ser Ala Val Leu Leu Met Val Ala Met<br>50 55 60<br>Tyr Leu Pro Ser Pro Leu Ala Phe Ser Ser Phe Pro Ser Val Leu Leu<br>65 70 75 80<br>Val Thr Thr Leu Phe Arg Leu Gly Ile Ser Ile Ala Thr Thr Arg Leu<br>85 90 95<br>Ile Leu Leu Gln Gly Asp Ala Gly His Ile Ile Glu Thr Phe Gly Asn<br>100 105 110<br>Phe Val Val Gly Gly Asn Leu Ile Val Gly Leu Val Val Phe Leu Ile<br>115 120 125<br>Leu Thr Ile Val Gln Phe Val Val Ile Thr Lys Gly Ala Glu Arg Val<br>130 135 140<br>Ala Glu Val Ala Ala Arg Phe Ser Leu Asp Ala Met Pro Gly Lys Gln<br>145 150 155 160<br>Met Ser Ile Asp Ala Asp Leu Arg Ala Gly Thr Ile Asp Met Asp Glu<br>165 170 175<br>Ala Arg Arg Arg Arg Arg Thr Val Glu Lys Glu Ser Gln Leu Tyr Gly<br>180 185 190<br>Ala Met Asp Gly Ala Met Lys Phe Val Lys Gly Asp Ala Ile Ala Gly<br>195 200 205<br>Leu Ile Ile Val Ala Val Asn Leu Leu Gly Gly Met Leu Val Gly Val<br>210 215 220<br>Leu Gln Arg Gly Leu Ser Ala Gly Glu Ala Val Gln Thr Tyr Ala Ile<br>225 230 235 240<br>Leu Thr Ile Gly Asp Gly Leu Ile Ala Gln Ile Pro Ala Leu Phe Ile<br>245 250 255 |      |

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Ala | Ile | Cys | Ala | Gly | Ile | Ile | Val | Thr | Arg | Val | Gln | Thr | Gly | Asp | Gly |
|    |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
|    | Pro | Ser | Asn | Val | Gly | Thr | Asp | Ile | Gly | Ala | Gln | Val | Leu | Ala | Gln | Pro |
|    |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| 5  | Arg | Ala | Leu | Val | Ile | Ala | Gly | Ala | Ile | Ser | Ala | Gly | Leu | Gly | Leu | Ile |
|    |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
|    | Pro | Gly | Met | Pro | Thr | Leu | Val | Phe | Phe | Ala | Leu | Ala | Ala | Ala | Val | Gly |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     | 320 |     |
| 10 | Thr | Ile | Gly | Phe | Val | Leu | Leu | Arg | Ala | Ser | Gln | Arg | Pro | Pro | Glu | Gly |
|    |     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
|    | Ala | Glu | Pro | Ala | Leu | Ala | Gly | Met | Ala | Ala | Asp | Gly | Gln | Pro | Arg | Thr |
|    |     |     |     | 340 |     |     |     | 345 |     |     |     |     |     | 350 |     |     |
|    | Arg | Ala | Pro | Ala | Asp | Gly | Gln | Ala | Glu | Phe | Ala | Pro | Thr | Val | Pro | Leu |
|    |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| 15 | Ile | Ile | Asp | Val | Ala | Ala | Arg | Leu | Gln | Pro | Arg | Phe | Glu | Pro | Ala | Thr |
|    |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
|    | Leu | Thr | Asp | Asp | Leu | Leu | Gln | Ile | Arg | Arg | Ala | Leu | Tyr | Phe | Asp | Leu |
|    | 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     | 400 |     |
| 20 | Gly | Val | Pro | Phe | Pro | Gly | Ile | Gln | Leu | Arg | Phe | Thr | Glu | Ala | Leu | Ala |
|    |     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |
|    | Ala | Asn | Thr | Tyr | Thr | Ile | Val | Leu | Ser | Glu | Ile | Pro | Val | Ala | Gln | Gly |
|    |     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
|    | Met | Leu | Arg | Asp | Asp | Ala | Val | Leu | Val | Arg | Asp | Thr | Glu | Gln | Asn | Leu |
|    |     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |
| 25 | Gln | Ala | Leu | Arg | Ile | Ala | Tyr | Glu | Thr | Gly | Ala | Ala | Phe | Leu | Pro | Asp |
|    |     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
|    | Thr | Pro | Thr | Ile | Trp | Val | Ala | Ala | Ser | Leu | Thr | Gly | Ala | Leu | Arg | Asp |
|    | 465 |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |     |
| 30 | Ala | Gly | Ile | Pro | Tyr | Leu | Gly | Ile | Ser | Gln | Ile | Leu | Thr | Trp | His | Leu |
|    |     |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |
|    | Ala | Tyr | Val | Leu | Lys | Lys | Tyr | Ser | Ala | Asp | Phe | Ile | Gly | Ile | Gln | Glu |
|    |     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |
|    | Thr | Arg | Phe | Leu | Leu | Ser | Ala | Met | Glu | Glu | Arg | Phe | Pro | Asp | Leu | Val |
|    |     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |
| 35 | Lys | Glu | Cys | Leu | Arg | Val | Met | Pro | Val | Gln | Lys | Ile | Ala | Glu | Ile | Leu |
|    |     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |
|    | Gln | Arg | Leu | Val | Ser | Glu | Glu | Val | Ser | Ile | Arg | Asn | Leu | Arg | Ala | Val |
|    | 545 |     |     |     |     | 550 |     |     |     | 555 |     |     |     |     | 560 |     |
| 40 | Leu | Glu | Ala | Leu | Val | Glu | Trp | Gly | Gln | Lys | Glu | Lys | Asp | Thr | Val | Leu |
|    |     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |
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    gag gcg ttg cgc cat att ctc gac gca ggc gct tcg atg ggg ggc ttg      96
    Glu Ala Leu Arg His Ile Leu Asp Ala Gly Ala Ser Met Gly Gly Leu
                                20                               25                               30

    cag ggg ttg gac gag gcg cag cag ggc ttg tac gcg atc ggt cat      144
    Gln Gly Leu Asp Glu Ala Gln Gln Ala Leu Tyr Ala Ile Gly His
                                35                               40                               45

    ggc gcc tac gaa cag ggg cgc tat gcc gac gcg ttg aaa atg ttc tgc      192
    Gly Ala Tyr Glu Gln Gly Arg Tyr Ala Asp Ala Leu Lys Met Phe Cys
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    ctg ctg gtc gcg tgc gat ccg ctg gaa gcc cgt tat ctg ctg gcc ctg      240
    Leu Leu Val Ala Cys Asp Pro Leu Glu Ala Arg Tyr Leu Leu Ala Leu
    65                               70                               75                               80

    ggc gcc gcg gcc cag gag ctg ggg ctg tac gag cat gcc ttg cag caa      288
    Gly Ala Ala Ala Gln Glu Leu Gly Leu Tyr Glu His Ala Leu Gln Gln
                                85                               90                               95

    tac gcg gcc gcg gcg gct ttg cag ttg gac tcc ccc agg ccc ctg ttg      336
    Tyr Ala Ala Ala Ala Ala Leu Gln Leu Asp Ser Pro Arg Pro Leu Leu
                                100                              105                              110

    cat ggc gcc gag tgc ctg tat gcg ttg ggt cgt cgc cgc gac gcc ctg      384
    His Gly Ala Glu Cys Leu Tyr Ala Leu Gly Arg Arg Arg Asp Ala Leu
                                115                              120                              125

    gat acg ctc gac atg gtg ctt gag ttg tgc ggc tcg ccg gag cgt gcg      432
    Asp Thr Leu Asp Met Val Leu Glu Leu Cys Gly Ser Pro Glu Arg Ala
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    gcc ctg cgc gaa cgg gcc gag ttg ctg cgc agg agc tat gca cgt gcc      480
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|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
|    | Arg | Ala | Val | Asp | Ala | Ala | Gly | Ala | Ser | Pro | Ser | Ala | Leu | Arg | Gln | Ala |      |
|    |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |      |
| 5  | ttg | ctg | caa | ctg | ggc | atc | ctg | gac | gaa | cgc | ttc | gga | tgg | gga | gag | ctg | 432  |
|    | Leu | Leu | Gln | Leu | Gly | Ile | Leu | Asp | Glu | Arg | Phe | Gly | Trp | Gly | Glu | Leu |      |
|    |     |     | 130 |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |      |
| 10 | ccg | gcg | caa | ggc | gtg | gcc | atg | gtg | tca | ggg | ccg | ccg | gcc | tat | gtc | gcg | 480  |
|    | Pro | Ala | Gln | Gly | Val | Ala | Met | Val | Ser | Gly | Pro | Pro | Ala | Tyr | Val | Ala |      |
|    | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |      |
| 15 | ctg | gtc | gag | cag | gcg | gta | gcg | gcg | ttg | ccc | aag | ggg | gcc | ggc | aat | cag | 528  |
|    | Leu | Val | Glu | Gln | Ala | Val | Ala | Ala | Leu | Pro | Lys | Gly | Ala | Gly | Asn | Gln |      |
|    |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |      |
| 20 | cag | gtg | gcg | gtg | ttt | cgc | ctc | aag | cat | gct | tcc | gtg | agc | gac | cgg | gtg | 576  |
|    | Gln | Val | Ala | Val | Phe | Arg | Leu | Lys | His | Ala | Ser | Val | Ser | Asp | Arg | Val |      |
|    |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |      |
| 25 | atc | cgt | tat | cga | gac | cag | cag | gta | ggt | acg | ccg | ggg | atg | gcc | acc | atg | 624  |
|    | Ile | Arg | Tyr | Arg | Asp | Gln | Gln | Val | Val | Thr | Pro | Gly | Met | ala | Thr | Met |      |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |      |
| 30 | ctg | cgc | caa | ttg | atc | ctg | ggg | gcg | ggg | ccg | ggc | aac | gac | gcg | gcg | ctg | 672  |
|    | Leu | Arg | Gln | Leu | Ile | Leu | Gly | Ala | Gly | Pro | Gly | Asn | Asp | Ala | Ala | Leu |      |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |      |
| 35 | gcc | gcg | gtg | gcg | gcg | ccg | ctg | cgg | gaa | aat | ccg | ccg | gtg | ttc | ggc | gat | 720  |
|    | Ala | Ala | Val | Ala | Ala | Pro | Leu | Arg | Glu | Asn | Pro | Pro | Val | Phe | Gly | Asp |      |
|    | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |      |
| 40 | gcg | gca | gct | gac | ggg | aac | gcg | ccg | ctc | gct | ggc | gca | gcc | cag | gca | gcc | 768  |
|    | Ala | Ala | Ala | Asp | Gly | Asn | Ala | Pro | Leu | Ala | Gly | Ala | Ala | Gln | Ala | Ala |      |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |      |
| 45 | ggc | cgg | cgc | ctg | agc | gag | ccc | agc | gtg | cag | gcc | gac | acg | cgc | ctc | aat | 816  |
|    | Gly | Arg | Arg | Leu | Ser | Glu | Pro | Ser | Val | Gln | Ala | Asp | Thr | Arg | Leu | Asn |      |
|    |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |      |
| 50 | gcc | ttg | atc | gtg | cag | gat | att | ccc | gaa | cgg | atg | cca | atc | tac | cgt | gcc | 864  |
|    | Ala | Leu | Ile | Val | Gln | Asp | Ile | Pro | Glu | Arg | Met | Pro | Ile | Tyr | Arg | Ala |      |
|    |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |      |
| 55 | ctg | atc | gag | cag | ttg | gat | gtg | ccc | agc | acc | ctg | atc | gaa | ata | gag | gcc | 912  |
|    | Leu | Ile | Glu | Gln | Leu | Asp | Val | Pro | Ser | Thr | Leu | Ile | Glu | Ile | Glu | Ala |      |
|    |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |      |
| 60 | atg | atc | gtg | gac | gtc | aat | acc | gat | ctg | gtc | aac | gag | ctg | ggt | gtc | acc | 960  |
|    | Met | Ile | Val | Asp | Val | Asn | Thr | Asp | Leu | Val | Asn | Glu | Leu | Gly | Val | Thr |      |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |      |
| 65 | tgg | ggg | gcg | cag | atc | gga | acc | acc | agc | ctg | ggc | tat | ggc | gat | ctg | ggg | 1008 |
|    | Trp | Gly | Ala | Gln | Ile | Gly | Thr | Thr | Ser | Leu | Gly | Tyr | Gly | Asp | Leu | Gly |      |
|    |     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |      |
| 70 | ctg | cgt | ccc | ggc | aac | ggc | ctg | ccc | gtg | gac | ggc | gcg | gcg | gcc | gac | ctg | 1056 |
|    | Leu | Arg | Pro | Gly | Asn | Gly | Leu | Pro | Val | Asp | Gly | Ala | Ala | Ala | Asp | Leu |      |
|    |     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |      |
| 75 | gcg | ccc | gga | acc | ttg | ggg | atc | agt | gtc | agt | acc | cgg | ctg | gcg | gcg | cgc | 1104 |

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|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
|    | Ala | Pro | Gly | Thr | Leu | Gly | Ile | Ser | Val | Ser | Thr | Arg | Leu | Ala | Ala | Arg |      |
|    |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |      |
| 5  | ttg | cgt | gcg | ttg | gag | tcg | gac | ggg | cag | gcc | aat | atc | ctg | tct | cag | ccg | 1152 |
|    | Leu | Arg | Ala | Leu | Glu | Ser | Asp | Gly | Gln | Ala | Asn | Ile | Leu | Ser | Gln | Pro |      |
|    |     |     | 370 |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |      |
| 10 | tcc | atc | ctg | acc | gcc | gac | aac | ctc | ggc | gcc | atg | ata | gac | ctg | tcg | gat | 1200 |
|    | Ser | Ile | Leu | Thr | Ala | Asp | Asn | Leu | Gly | Ala | Met | Ile | Asp | Leu | Ser | Asp |      |
|    |     |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |      |
| 15 | acc | ttc | tac | att | cgc | acc | ctg | ggc | gag | cgc | gta | gcg | aca | gtc | acg | cct | 1248 |
|    | Thr | Phe | Tyr | Ile | Arg | Thr | Leu | Gly | Glu | Arg | Val | Ala | Thr | Val | Thr | Pro |      |
|    |     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |      |
| 20 | gtc | acg | gtg | ggt | acg | tcg | ttg | cgt | gtg | acg | ccg | cgc | tat | atc | gcc | gcc | 1296 |
|    | Val | Thr | Val |     | Thr | Ser | Leu | Arg | Val | Thr | Pro | Arg | Tyr | Ile | Ala | Ala |      |
|    |     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |      |
| 25 | aag | gga | gga | cgc | cag | gtg | gaa | ttg | gcg | atc | gat | atc | gag | gac | gga | cgg | 1344 |
|    | Lys | Gly | Gly | Arg | Gln | Val | Glu | Leu | Ala | Ile | Asp | Ile | Glu | Asp | Gly | Arg |      |
|    |     |     | 435 |     |     |     | 440 |     |     |     |     |     | 445 |     |     |     |      |
| 30 | gtc | ttg | cag | gag | tat | ccc | atc | gat | ggt | ctg | ccc | cgg | gtt | cgg | aaa | agc | 1392 |
|    | Val | Leu | Gln | Glu | Tyr | Pro | Ile | Asp | Gly | Leu | Pro | Arg | Val | Arg | Lys | Ser |      |
|    |     |     | 450 |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |      |
| 35 | agc | atc | agc | acg | ctg | gcg | gtg | gtg | ggg | gac | gag | cag | acg | ctg | ctg | atc | 1440 |
|    | Ser | Ile | Ser | Thr | Leu | Ala | Val | Val | Gly | Asp | Glu | Gln | Thr | Leu | Leu | Ile |      |
|    |     |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |      |
| 40 | ggc | ggc | tac | aac | aat | cgc | cgt | gac | gaa | gag | gac | gtc | gag | aaa | gtg | ccg | 1488 |
|    | Gly | Gly | Tyr | Asn | Asn | Arg | Arg | Asp | Glu | Gln | Val | Glu | Lys | Val | Pro |     |      |
|    |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |     |      |
| 45 | ctg | ctg | gga | gat | atc | ccc | ggc | ctg | ggg | ttc | ttg | ttc | tcg | agc | aag | tcc | 1536 |
|    | Leu | Leu | Gly | Asp | Ile | Pro | Gly | Leu | Gly | Phe | Leu | Phe | Ser | Ser | Lys | Ser |      |
|    |     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |      |
| 50 | cgg | gcg | gta | cag | cgc | cgc | gag | cgg | ctg | ttc | ctg | atc | cgg | ccg | cgt | gtc | 1584 |
|    | Arg | Ala | Val | Gln | Arg | Arg | Glu | Arg | Leu | Phe | Leu | Ile | Arg | Pro | Arg | Val |      |
|    |     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |      |
| 55 | gtg | gct | atc | gag | ggc | aag | ccg | gtc | ttc | agc | ccc | gtt | gcg | ggc | acg | tcg | 1632 |
|    | Val | Ala | Ile | Glu | Gly | Lys | Pro | Val | Phe | Ser | Pro | Val | Ala | Gly | Thr | Ser |      |
|    |     |     | 530 |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |      |
| 60 | cag | gtg | ttc | atg | agc | acg | ggt | tgg | ggc | ggg | cat | ggc | agc | agc | ctg | agc | 1680 |
|    | Gln | Val | Phe | Met | Ser | Thr | Gly | Trp | Gly | Gly | His | Gly | Ser | Ser | Leu | Ser |      |
|    |     |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |      |
| 65 | att | gca | ccc | ggc | gag | ggc | ggg | cat | aca | caa | gtg | cgt | cat | gat | gcc | cgg | 1728 |
|    | Ile | Ala | Pro | Gly | Glu | Gly | Gly | His | Thr | Gln | Val | Arg | His | Asp | Ala | Arg |      |
|    |     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |      |
| 70 | gcg | ggc | agg | ccg | gtc | cgg | ctg | gtg | ccg | gat | tca | ttg | cat | gtg | gag | tat | 1776 |
|    | Ala | Gly | Arg | Pro | Val | Arg | Leu | Val | Pro | Asp | Ser | Leu | His | Val | Glu | Tyr |      |
|    |     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |      |
| 75 | ggc | gag | gcg | ggg | gag | gcg | tcg | ccc | tga |     |     |     |     |     |     |     | 1803 |

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Gly Glu Ala Gly Glu Ala Ser Pro \*  
595 600

5 <210> 6  
<211> 600  
<212> PRT  
<213> Bordetella pertussis

10 <400> 6

Met ala Ile Gly Arg Leu Gly Tyr Leu Val Arg Gly Ala Trp Ala Gly  
1 5 10 15  
Gly Val Met Leu Leu Ala Ala Gly Ser Ala Trp Ala Ala Pro Asn Trp  
20 25 30  
15 Pro Leu Ala Pro Tyr Ser Tyr Tyr Ala Gln Gln Gln Ser Leu Ser Asp  
35 40 45  
Val Leu Arg Glu Phe Ala Ala Gly Phe Ser Leu Ala Leu Gln Gln Gly  
50 55 60  
20 Lys Gly Val Gln Gly Val Val Asn Gly Arg Phe Asn Ala Arg Thr Pro  
65 70 75 80  
Thr Glu Phe Ile Glu Arg Leu Ser Gly Ile Tyr Gly Phe Asn Trp Phe  
85 90 95  
Val His Ala Gly Thr Leu Tyr Val Ser Arg Thr Ser Asp Val Val Thr  
100 105 110  
25 Arg Ala Val Asp Ala Ala Gly Ala Ser Pro Ser Ala Leu Arg Gln Ala  
115 120 125  
Leu Leu Gln Leu Gly Ile Leu Asp Glu Arg Phe Gly Trp Gly Glu Leu  
130 135 140  
30 Pro Ala Gln Gly Val Ala Met Val Ser Gly Pro Pro Ala Tyr Val Ala  
145 150 155 160  
Leu Val Glu Gln Ala Val Ala Ala Leu Pro Lys Gly Ala Gly Asn Gln  
165 170 175  
Gln Val Ala Val Phe Arg Leu Lys His Ala Ser Val Ser Asp Arg Val  
180 185 190  
35 Ile Arg Tyr Arg Asp Gln Gln Val Val Thr Pro Gly Met ala Thr Met  
195 200 205  
Leu Arg Gln Leu Ile Leu Gly Ala Gly Pro Gly Asn Asp Ala Ala Leu  
210 215 220  
40 Ala Ala Val Ala Ala Pro Leu Arg Glu Asn Pro Pro Val Phe Gly Asp  
225 230 235 240  
Ala Ala Ala Asp Gly Asn Ala Pro Leu Ala Gly Ala Ala Gln Ala Ala  
245 250 255  
Gly Arg Arg Leu Ser Glu Pro Ser Val Gln Ala Asp Thr Arg Leu Asn  
260 265 270  
45 Ala Leu Ile Val Gln Asp Ile Pro Glu Arg Met Pro Ile Tyr Arg Ala  
275 280 285  
Leu Ile Glu Gln Leu Asp Val Pro Ser Thr Leu Ile Glu Ile Glu Ala  
290 295 300  
50 Met Ile Val Asp Val Asn Thr Asp Leu Val Asn Glu Leu Gly Val Thr  
305 310 315 320  
Trp Gly Ala Gln Ile Gly Thr Thr Ser Leu Gly Tyr Gly Asp Leu Gly  
325 330 335  
Leu Arg Pro Gly Asn Gly Leu Pro Val Asp Gly Ala Ala Ala Asp Leu  
340 345 350  
55 Ala Pro Gly Thr Leu Gly Ile Ser Val Ser Thr Arg Leu Ala Ala Arg  
355 360 365  
Leu Arg Ala Leu Glu Ser Asp Gly Gln Ala Asn Ile Leu Ser Gln Pro  
370 375 380  
60 Ser Ile Leu Thr Ala Asp Asn Leu Gly Ala Met Ile Asp Leu Ser Asp  
385 390 395 400



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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Thr                        | Phe | Tyr | Ile | Arg | Thr | Leu | Gly | Glu | Arg | Val | Ala | Thr | Val | Thr | Pro |     |
|    |                            |     |     | 405 |     |     |     |     |     | 410 |     |     |     |     | 415 |     |     |
|    | Val                        | Thr | Val | Gly | Thr | Ser | Leu | Arg | Val | Thr | Pro | Arg | Tyr | Ile | Ala | Ala |     |
|    |                            |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |     |
| 5  | Lys                        | Gly | Gly | Arg | Gln | Val | Glu | Leu | Ala | Ile | Asp | Ile | Glu | Asp | Gly | Arg |     |
|    |                            |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |     |
|    | Val                        | Leu | Gln | Glu | Tyr | Pro | Ile | Asp | Gly | Leu | Pro | Arg | Val | Arg | Lys | Ser |     |
|    |                            | 450 |     |     |     | 455 |     |     |     |     |     | 460 |     |     |     |     |     |
| 10 | Ser                        | Ile | Ser | Thr | Leu | Ala | Val | Val | Gly | Asp | Glu | Gln | Thr | Leu | Leu | Ile |     |
|    | 465                        |     |     |     | 470 |     |     |     |     |     | 475 |     |     |     |     | 480 |     |
|    | Gly                        | Gly | Tyr | Asn | Asn | Arg | Arg | Asp | Glu | Glu | Gln | Val | Glu | Lys | Val | Pro |     |
|    |                            |     |     | 485 |     |     |     |     | 490 |     |     |     |     |     | 495 |     |     |
|    | Leu                        | Leu | Gly | Asp | Ile | Pro | Gly | Leu | Gly | Phe | Leu | Phe | Ser | Ser | Lys | Ser |     |
|    |                            |     | 500 |     |     |     |     | 505 |     |     |     |     |     | 510 |     |     |     |
| 15 | Arg                        | Ala | Val | Gln | Arg | Arg | Glu | Arg | Leu | Phe | Leu | Ile | Arg | Pro | Arg | Val |     |
|    |                            | 515 |     |     |     |     | 520 |     |     |     |     |     | 525 |     |     |     |     |
|    | Val                        | Ala | Ile | Glu | Gly | Lys | Pro | Val | Phe | Ser | Pro | Val | Ala | Gly | Thr | Ser |     |
|    |                            | 530 |     |     |     | 535 |     |     |     |     |     | 540 |     |     |     |     |     |
| 20 | Gln                        | Val | Phe | Met | Ser | Thr | Gly | Trp | Gly | Gly | His | Gly | Ser | Ser | Leu | Ser |     |
|    | 545                        |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |     |
|    | Ile                        | Ala | Pro | Gly | Glu | Gly | Gly | His | Thr | Gln | Val | Arg | His | Asp | Ala | Arg |     |
|    |                            |     |     | 565 |     |     |     |     | 570 |     |     |     |     |     | 575 |     |     |
|    | Ala                        | Gly | Arg | Pro | Val | Arg | Leu | Val | Pro | Asp | Ser | Leu | His | Val | Glu | Tyr |     |
|    |                            |     | 580 |     |     |     |     | 585 |     |     |     |     |     | 590 |     |     |     |
| 25 | Gly                        | Glu | Ala | Gly | Glu | Ala | Ser | Pro |     |     |     |     |     |     |     |     |     |
|    |                            |     | 595 |     |     |     |     | 600 |     |     |     |     |     |     |     |     |     |
|    | <210> 7                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 1281                 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 30 | <212> DNA                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <220>                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <221> CDS                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 35 | <222> (1)...(1281)         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <400> 7                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | atg                        | acg | acg | gcg | ctg | gaa | ttc | cgc | gtg | ctt | tca | ggc | gca | cag | tgc | atg | 48  |
| 40 | Met                        | Thr | Thr | Ala | Leu | Glu | Phe | Arg | Val | Leu | Ser | Gly | Ala | Gln | Cys | Met |     |
|    | 1                          |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|    | gcg                        | cgc | tgc | ccg | gcc | gtg | cat | ggc | gcg | cgc | gtg | ggc | gcc | aat | ccg | cat | 96  |
|    | Ala                        | Arg | Cys | Pro | Ala | Val | His | Gly | Ala | Arg | Val | Gly | Ala | Asn | Pro | His |     |
|    |                            |     |     | 20  |     |     |     | 25  |     |     |     |     |     | 30  |     |     |     |
| 45 | tgc                        | gat | atc | gtc | ctg | acc | ggc | gag | gac | atg | ccc | gaa | gtg | gcg | gga | tgg | 144 |
|    | Cys                        | Asp | Ile | Val | Leu | Thr | Gly | Glu | Asp | Met | Pro | Glu | Val | Ala | Gly | Trp |     |
|    |                            |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |     |
| 50 | ctg                        | gag | atc | gac | cag | tcc | ggc | tgg | cgg | ttg | gcc | ggc | gcc | gtg | acg | ccc | 192 |
|    | Leu                        | Glu | Ile | Asp | Gln | Ser | Gly | Trp | Arg | Leu | Ala | Gly | Ala | Val | Thr | Pro |     |
|    |                            | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |
| 55 | ggc                        | ctg | gac | gcc | cag | gcg | ccg | tgt | ccg | ccc | gcg | gcc | ttc | aac | gaa | ccc | 240 |
|    | Gly                        | Leu | Asp | Ala | Gln | Ala | Pro | Cys | Pro | Pro | Ala | Ala | Phe | Asn | Glu | Pro |     |
|    |                            | 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
|    | gta                        | gag | ctg | gga | gcc | gcc | tgg | atc | acc | gtg | gcc | gcc | cct | tcc | gcg | ccg | 288 |
| 60 | Val                        | Glu | Leu | Gly | Ala | Ala | Trp | Ile | Thr | Val | Ala | Ala | Pro | Ser | Ala | Pro |     |
|    |                            |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     |     | 95  |     |

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|    |  |      |
|----|--|------|
| 5  | tgg ccc gcg ccg ccg gag ccg tgc ggc ccg gac ggc agc gac aca gcc<br>Trp Pro Ala Pro Pro Glu Pro Cys Gly Pro Asp Gly Ser Asp Thr Ala | 336  |
|    | 100 105 110  |      |
| 10 | ttg cac gac gtc cct ggc tgc aca agc ccg ccg tcc gtc gct gcc ctc<br>Leu His Asp Val Pro Gly Ser Thr Ser Pro Pro Ser Val Ala Ala Leu | 384  |
|    | 115 120 125  |      |
| 15 | atg ccg cgc cga cgt gca gga cgg ccc tgg ctg gcg ctg ggc gcg gcc<br>Met Pro Arg Arg Arg Ala Gly Arg Pro Trp Leu Ala Leu Gly Ala Ala | 432  |
|    | 130 135 140  |      |
| 20 | gcg gcc gtc ctg ctg gtc ggc ctg gcc acg gcg ctg gtt tcc gtg acc<br>Ala Ala Val Leu Leu Val Gly Leu Ala Thr Ala Leu Val Ser Val Thr | 480  |
|    | 145 150 155 160  |      |
| 25 | aca ccc gcc acg ccg ccg gcc gcg ccg ccc cca acg ccc acc gcg ccg<br>Thr Pro Ala Thr Pro Pro Ala Ala Pro Pro Pro Thr Pro Thr Ala Pro | 528  |
|    | 165 170 175  |      |
| 30 | ctg gtc cgc gcc gcg gcg ctc atc gac agc ctg ggc ctt acc gag caa<br>Leu Val Arg Ala Ala Leu Ile Asp Ser Leu Gly Leu Thr Glu Gln     | 576  |
|    | 180 185 190  |      |
| 35 | tta caa gcg gcc tac ggc cgt ggc ggc gtg ctc acc gtg acc gga tgg<br>Leu Gln Ala Ala Tyr Gly Arg Gly Gly Val Leu Thr Val Thr Gly Trp | 624  |
|    | 195 200 205  |      |
| 40 | gtg cac gac gag acg gaa ttc gct cgg gtc gcc agg gcg ttg gcg caa<br>Val His Asp Glu Thr Glu Phe Ala Arg Val Ala Arg Ala Leu Ala Gln | 672  |
|    | 210 215 220  |      |
| 45 | ctt gcg cca ccg cct gcc atg cag gta agc agg cag gac gag gcc agg<br>Leu Ala Pro Arg Pro Ala Met Gln Val Ser Arg Gln Asp Glu Ala Arg | 720  |
|    | 225 230 235 240  |      |
| 50 | gcc ctg gcc tgc gat gtc ctg gcg aca ttc ggg gtg cgc tac atg gcg<br>Ala Leu Ala Cys Asp Val Leu Ala Thr Phe Gly Val Arg Tyr Met ala | 768  |
|    | 245 250 255  |      |
| 55 | cgc ccg tac ggc aat ggc cgc ctg gcg atc tgc ggc atc gcc agc gat<br>Arg Pro Tyr Gly Asn Gly Arg Leu Ala Ile Ser Gly Ile Ala Ser Asp | 816  |
|    | 260 265 270  |      |
| 60 | gcg cac gaa cgc gcc gcg gcg ctg cat gcg gtg cgc atg cgc ctg ccg<br>Ala His Glu Arg Ala Ala Ala Leu His Ala Val Arg Met Arg Leu Pro | 864  |
|    | 275 280 285  |      |
| 65 | ggc atg acg atc ctc ggt cgc gat gta cgc ctg gcc gac gag gtc tgc<br>Gly Met Thr Ile Leu Gly Arg Asp Val Arg Leu Ala Asp Glu Val Ser | 912  |
|    | 290 295 300  |      |
| 70 | gcc cag ttc gcg gcc cag ctg gcc gac gaa cgc ctc gac ggc gtc aag<br>Ala Gln Phe Ala Ala Gln Leu Ala Asp Glu Arg Leu Asp Gly Val Lys | 960  |
|    | 305 310 315 320  |      |
| 75 | ctc agc tgg cac gcc gac cgc ctg gac gca gat ccc ggc gga ttg gcg<br>Leu Ser Trp His Ala Asp Arg Leu Asp Ala Asp Pro Gly Gly Leu Ala | 1008 |
|    | 325 330 335  |      |

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5 gca ggc cgc atg gcg cgc ctg cgc gag ctg gtg gcc gcg ttc aac cag 1056  
Ala Gly Arg Met ala Arg Leu Arg Glu Leu Val Ala Ala Phe Asn Gln  
340 345 350

5 cgc aac tac gac gtc gtc cgg ctg ccg gcc acc gcc gcg cgc gcg acg 1104  
Arg Asn Tyr Asp Val Val Arg Leu Pro Ala Thr Ala Ala Arg Ala Thr  
355 360 365

10 cgg gat cac gtg ccg ttc gag ata cgc agt gtc gtg agc ggc ccg caa 1152  
Arg Asp His Val Pro Phe Glu Ile Arg Ser Val Val Ser Gly Pro Gln  
370 375 380

15 ccg tac ctg atg ctg gcc gat ggc agc cgc ctc ctg gtg ggc gga ctg 1200  
Pro Tyr Leu Met Leu Ala Asp Gly Ser Arg Leu Leu Val Gly Gly Leu  
385 390 395 400

20 cgg gac cag tat cgc ctt acc gcc atc gaa tcc ggc cgc ctg gtc ttc 1248  
Arg Asp Gln Tyr Arg Leu Thr Ala Ile Glu Ser Gly Arg Leu Val Phe  
405 410 415

25 gat ggt ccc gaa ccg gtc atc gtg acg cga tga 1281  
Asp Gly Pro Glu Pro Val Ile Val Thr Arg \*  
420 425

30 <210> 8  
<211> 426  
<212> PRT  
<213> Bordetella pertussis

<400> 8

35 Met Thr Thr Ala Leu Glu Phe Arg Val Leu Ser Gly Ala Gln Cys Met  
1 5 10 15  
Ala Arg Cys Pro Ala Val His Gly Ala Arg Val Gly Ala Asn Pro His  
20 25 30

40 Cys Asp Ile Val Leu Thr Gly Glu Asp Met Pro Glu Val Ala Gly Trp  
35 40 45  
Leu Glu Ile Asp Gln Ser Gly Trp Arg Leu Ala Gly Ala Val Thr Pro  
50 55 60  
Gly Leu Asp Ala Gln Ala Pro Cys Pro Pro Ala Ala Phe Asn Glu Pro  
65 70 75 80  
Val Glu Leu Gly Ala Ala Trp Ile Thr Val Ala Ala Pro Ser Ala Pro  
85 90 95

45 Trp Pro Ala Pro Pro Glu Pro Cys Gly Pro Asp Gly Ser Asp Thr Ala  
100 105 110  
Leu His Asp Val Pro Gly Ser Thr Ser Pro Pro Ser Val Ala Ala Leu  
115 120 125

50 Met Pro Arg Arg Arg Ala Gly Arg Pro Trp Leu Ala Leu Gly Ala Ala  
130 135 140  
Ala Ala Val Leu Leu Val Gly Leu Ala Thr Ala Leu Val Ser Val Thr  
145 150 155 160  
Thr Pro Ala Thr Pro Pro Ala Ala Pro Pro Thr Pro Thr Ala Pro  
165 170 175

55 Leu Val Arg Ala Ala Ala Leu Ile Asp Ser Leu Gly Leu Thr Glu Gln  
180 185 190  
Leu Gln Ala Ala Tyr Gly Arg Gly Gly Val Leu Thr Val Thr Gly Trp  
195 200 205

60 Val His Asp Glu Thr Glu Phe Ala Arg Val Ala Arg Ala Leu Ala Gln  
210 215 220

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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Leu                        | Ala | Pro | Arg | Pro | Ala | Met | Gln | Val | Ser | Arg | Gln | Asp | Glu | Ala | Arg |     |
|    | 225                        |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |
|    | Ala                        | Leu | Ala | Cys | Asp | Val | Leu | Ala | Thr | Phe | Gly | Val | Arg | Tyr | Met | ala |     |
|    |                            |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |     |
| 5  | Arg                        | Pro | Tyr | Gly | Asn | Gly | Arg | Leu | Ala | Ile | Ser | Gly | Ile | Ala | Ser | Asp |     |
|    |                            |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |     |
|    | Ala                        | His | Glu | Arg | Ala | Ala | Ala | Leu | His | Ala | Val | Arg | Met | Arg | Leu | Pro |     |
|    |                            |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |     |
| 10 | Gly                        | Met | Thr | Ile | Leu | Gly | Arg | Asp | Val | Arg | Leu | Ala | Asp | Glu | Val | Ser |     |
|    |                            | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |     |
|    | Ala                        | Gln | Phe | Ala | Ala | Gln | Leu | Ala | Asp | Glu | Arg | Leu | Asp | Gly | Val | Lys |     |
|    | 305                        |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |     |
|    | Leu                        | Ser | Trp | His | Ala | Asp | Arg | Leu | Asp | Ala | Asp | Pro | Gly | Gly | Leu | Ala |     |
|    |                            |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |     |
| 15 | Ala                        | Gly | Arg | Met | ala | Arg | Leu | Arg | Glu | Leu | Val | Ala | Ala | Phe | Asn | Gln |     |
|    |                            |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |     |
|    | Arg                        | Asn | Tyr | Asp | Val | Val | Arg | Leu | Pro | Ala | Thr | Ala | Ala | Arg | Ala | Thr |     |
|    |                            |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |     |
| 20 | Arg                        | Asp | His | Val | Pro | Phe | Glu | Ile | Arg | Ser | Val | Val | Ser | Gly | Pro | Gln |     |
|    |                            | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |     |
|    | Pro                        | Tyr | Leu | Met | Leu | Ala | Asp | Gly | Ser | Arg | Leu | Leu | Val | Gly | Gly | Leu |     |
|    | 385                        |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |     |
|    | Arg                        | Asp | Gln | Tyr | Arg | Leu | Thr | Ala | Ile | Glu | Ser | Gly | Arg | Leu | Val | Phe |     |
|    |                            |     |     | 405 |     |     |     |     |     | 410 |     |     |     |     | 415 |     |     |
| 25 | Asp                        | Gly | Pro | Glu | Pro | Val | Ile | Val | Thr | Arg |     |     |     |     |     |     |     |
|    |                            |     | 420 |     |     |     |     |     | 425 |     |     |     |     |     |     |     |     |
|    | <210> 9                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 300                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 30 | <212> DNA                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <220>                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <221> CDS                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 35 | <222> (1)...(300)          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <400> 9                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 40 | atg                        | agt | aca | tct | gtt | cta | gcc | ctg | acc | gaa | ctg | gaa | gtg | cgc | ctg | gca | 48  |
|    | Met                        | Ser | Thr | Ser | Val | Leu | Ala | Leu | Thr | Glu | Leu | Glu | Val | Arg | Leu | Ala |     |
|    | 1                          |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|    | tcg                        | ccg | ggc | ggt | tcc | gcc | ttg | cgc | gac | acc | ttg | ctg | tcg | cag | ctt | ggc | 96  |
|    | Ser                        | Pro | Gly | Gly | Ser | Ala | Leu | Arg | Asp | Thr | Leu | Leu | Ser | Gln | Leu | Gly |     |
| 45 |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
|    | gaa                        | ctg | gag | aca | cgt | ttg | cgc | gtc | cgc | ctg | cac | gat | ggc | gtg | ggg | cgc | 144 |
|    | Glu                        | Leu | Glu | Thr | Arg | Leu | Arg | Val | Arg | Leu | His | Asp | Gly | Val | Gly | Arg |     |
|    |                            |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |     |
| 50 | gac                        | acc | tat | ccc | gta | tgg | cgc | gac | gcg | ctg | gcg | gcc | gcc | acc | gcg | gcc | 192 |
|    | Asp                        | Thr | Tyr | Pro | Val | Trp | Arg | Asp | Ala | Leu | Ala | Ala | Ala | Thr | Ala | Ala |     |
|    |                            | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |
| 55 | cgg                        | cag | gta | ttg | ctg | cag | cgc | ccg | acc | ggg | ccg | gac | aac | cct | ccg | gcg | 240 |
|    | Arg                        | Gln | Val | Leu | Leu | Gln | Arg | Pro | Thr | Gly | Pro | Asp | Asn | Pro | Pro | Ala |     |
|    | 65                         |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
| 60 | tca                        | gtc | ttg | acg | cgc | ctg | agc | aat | gaa | caa | tgc | gcc | gaa | gga | gac | aag | 288 |
|    | Ser                        | Val | Leu | Thr | Arg | Leu | Ser | Asn | Glu | Gln | Cys | Ala | Glu | Gly | Asp | Lys |     |
|    |                            |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |

|    |   |     |
|----|---|-----|
|    | cat ggc cat taa   |     |
|    | His Gly His *   | 300 |
| 5  |   |     |
|    | <210> 10  |     |
|    | <211> 99  |     |
|    | <212> PRT   |     |
| 10 | <213> Bordetella pertussis                                      |     |
|    | <400> 10  |     |
|    | Met Ser Thr Ser Val Leu Ala Leu Thr Glu Leu Glu Val Arg Leu Ala |     |
|    | 1 5 10 15   |     |
| 15 | Ser Pro Gly Gly Ser Ala Leu Arg Asp Thr Leu Leu Ser Gln Leu Gly |     |
|    | 20 25 30  |     |
|    | Glu Leu Glu Thr Arg Leu Arg Val Arg Leu His Asp Gly Val Gly Arg |     |
|    | 35 40 45  |     |
| 20 | Asp Thr Tyr Pro Val Trp Arg Asp Ala Leu Ala Ala Ala Thr Ala Ala |     |
|    | 50 55 60  |     |
|    | Arg Gln Val Leu Leu Gln Arg Pro Thr Gly Pro Asp Asn Pro Pro Ala |     |
|    | 65 70 75 80   |     |
|    | Ser Val Leu Thr Arg Leu Ser Asn Glu Gln Cys Ala Glu Gly Asp Lys |     |
|    | 85 90 95  |     |
| 25 | His Gly His   |     |
|    | <210> 11  |     |
|    | <211> 267   |     |
|    | <212> DNA   |     |
| 30 | <213> Bordetella pertussis                                      |     |
|    | <220>   |     |
|    | <221> CDS   |     |
| 35 | <222> (1)...(267)   |     |
|    | <400> 11  |     |
|    | atg gcc att aac ctg gga ggc gac gca ggc cga gtg acc atg cag agc | 48  |
| 40 | Met ala Ile Asn Leu Gly Gly Asp Ala Gly Arg Val Thr Met Gln Ser |     |
|    | 1 5 10 15   |     |
|    | gtc aac cag gcg gtc aat acg cgg ctg aac gct cac gaa cgc gac ctg | 96  |
|    | Val Asn Gln Ala Val Asn Thr Arg Leu Asn Ala His Glu Arg Asp Leu |     |
|    | 20 25 30  |     |
| 45 | cgc agc cgc ctg gag gcg ctc agc gcg cgc gga gac ggc gcg gtc agc | 144 |
|    | Arg Ser Arg Leu Glu Ala Leu Ser Ala Arg Gly Asp Gly Ala Val Ser |     |
|    | 35 40 45  |     |
| 50 | acg tcc gac ctg ctg atc gtg caa cag gaa atg caa tcg tgg gtc gtg | 192 |
|    | Thr Ser Asp Leu Leu Ile Val Gln Gln Glu Met Gln Ser Trp Val Val |     |
|    | 50 55 60  |     |
| 55 | atg atc gat cta cag agc acg gtg gtc aag cag gtc gcg gat tcg ctc | 240 |
|    | Met Ile Asp Leu Gln Ser Thr Val Val Lys Gln Val Ala Asp Ser Leu |     |
|    | 65 70 75 80   |     |
|    | aag ggc gtc ata cag aag gcg agt tga                             | 267 |
| 60 | Lys Gly Val Ile Gln Lys Ala Ser *                               |     |
|    | 85  |     |

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5 <210> 12  
 <211> 88  
 <212> PRT  
 <213> Bordetella pertussis

10 <400> 12  
 Met ala Ile Asn Leu Gly Gly Asp Ala Gly Arg Val Thr Met Gln Ser  
 1 5 10 15  
 Val Asn Gln Ala Val Asn Thr Arg Leu Asn Ala His Glu Arg Asp Leu  
 20 25 30  
 Arg Ser Arg Leu Glu Ala Leu Ser Ala Arg Gly Asp Gly Ala Val Ser  
 35 40 45  
 15 Thr Ser Asp Leu Leu Ile Val Gln Gln Glu Met Gln Ser Trp Val Val  
 50 55 60  
 Met Ile Asp Leu Gln Ser Thr Val Val Lys Gln Val Ala Asp Ser Leu  
 65 70 75 80  
 20 Lys Gly Val Ile Gln Lys Ala Ser  
 85

25 <210> 13  
 <211> 327  
 <212> DNA  
 <213> Bordetella pertussis

30 <220>  
 <221> CDS  
 <222> (1)...(327)

35 <400> 13  
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 Met ala Asp Gln Ala Arg Phe Glu Leu Ala Leu Gly Glu Met Pro Gly  
 1 5 10 15  
 35 gca tcg gcc ccg aac ggg gcg atc gcc ctg gcg ccg gtc gcg ctc gac 96  
 Ala Ser Ala Pro Asn Gly Ala Ile Ala Leu Ala Pro Val Ala Leu Asp  
 20 25 30  
 40 gag ccg ctg ggc cgt cgc att ctt gga cag ttg cgc ggc ggc ctg gcc 144  
 Glu Pro Leu Gly Arg Arg Ile Leu Gly Gln Leu Arg Gly Gly Leu Ala  
 35 40 45  
 45 gat gtg gca gga aaa tgg cgg gcg gtg cag acg ggc ttg gcc gag gtg 192  
 Asp Val Ala Gly Lys Trp Arg Ala Val Gln Thr Gly Leu Ala Glu Val  
 50 55 60  
 50 agc cag gcg cct acc gtg gtg ggt atg ctc gat ctg cag gcc agg ttg 240  
 Ser Gln Ala Pro Thr Val Val Gly Met Leu Asp Leu Gln Ala Arg Leu  
 65 70 75 80  
 55 cta cag gca tcc gtg gag tac gag ttg gtg ggc aag gca ata ggg cgc 288  
 Leu Gln Ala Ser Val Glu Tyr Glu Leu Val Gly Lys Ala Ile Gly Arg  
 85 90 95  
 gcc acc caa aac gtc gat acg ctg gcg aga atg tca tga 327  
 Ala Thr Gln Asn Val Asp Thr Leu Ala Arg Met Ser \*  
 100 105

60

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<210> 14  
 <211> 108  
 <212> PRT  
 <213> Bordetella pertussis  
 5  
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 1 5 10 15  
 10 Ala Ser Ala Pro Asn Gly Ala Ile Ala Leu Ala Pro Val Ala Leu Asp  
 20 25 30  
 Glu Pro Leu Gly Arg Arg Ile Leu Gly Gln Leu Arg Gly Gly Leu Ala  
 35 40 45  
 Asp Val Ala Gly Lys Trp Arg Ala Val Gln Thr Gly Leu Ala Glu Val  
 50 55 60  
 15 Ser Gln Ala Pro Thr Val Val Gly Met Leu Asp Leu Gln Ala Arg Leu  
 65 70 75 80  
 Leu Gln Ala Ser Val Glu Tyr Glu Leu Val Gly Lys Ala Ile Gly Arg  
 85 90 95  
 20 Ala Thr Gln Asn Val Asp Thr Leu Ala Arg Met Ser  
 100 105  
 <210> 15  
 <211> 825  
 <212> DNA  
 <213> Bordetella pertussis  
 25  
 <220>  
 <221> CDS  
 <222> (1)...(825)  
 30  
 <400> 15  
 atg aac gcc atc ggg gcg atc caa cgg tat cgg cgc gcc gcg gga tgg 48  
 Met Asn Ala Ile Gly Ala Ile Gln Arg Tyr Arg Arg Gly Ala Gly Trp  
 1 5 10 15  
 35 gcg gcc ctg gtg ctc gcc ctg gcg ctg ctg gcc gcc tgc ggt gcc cgc 96  
 Ala Ala Leu Val Leu Ala Leu Ala Leu Ala Gly Cys Gly Ala Arg  
 20 25 30  
 40 gtc gag ctg ttg ggc gcg gcg ccc gag aac gaa gcc aac gaa gta ttg 144  
 Val Glu Leu Leu Gly Ala Ala Pro Glu Asn Glu Ala Asn Glu Val Leu  
 35 40 45  
 45 gcg gcg ctg ctc gag gca gcc atc gct gcg cag aag cag tcc gcc aag 192  
 Ala Ala Leu Leu Glu Ala Gly Ile Ala Ala Gln Lys Gln Ser Gly Lys  
 50 55 60  
 50 gcc gcc tac gcg gtt tgc gtg ccg gcc gag gcg gtg gcc cgg tgc ctg 240  
 Ala Gly Tyr Ala Val Ser Val Pro Ala Glu Ala Val Ala Arg Ser Leu  
 65 70 75 80  
 55 gag atc ctg cgc gca agc gcc ctg ccc cgc gag cag ttc gac gga atg 288  
 Glu Ile Leu Arg Ala Ser Gly Leu Pro Arg Glu Gln Phe Asp Gly Met  
 85 90 95  
 60 gga cgc ata ttc cgc aag gaa gcc ctg gtt tca tct ccg ctc gaa gag 336  
 Gly Arg Ile Phe Arg Lys Glu Gly Leu Val Ser Ser Pro Leu Glu Glu  
 100 105 110  
 60 cgc gcc cgc tac att tat gcg ctg tct cag gaa ttg gcc gac acc ctg 384

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|    |       |                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|-------|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Arg   | Ala                  | Arg | Tyr | Ile | Tyr | Ala | Leu | Ser | Gln | Glu | Leu | Ala | Asp | Thr | Leu |     |
|    |       |                      | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
| 5  | tcg   | cag                  | atc | gac | ggc | gtg | ctc | agc | gcc | cgc | gtg | cac | gtg | gtg | ctt | ccc | 432 |
|    | Ser   | Gln                  | Ile | Asp | Gly | Val | Leu | Ser | Ala | Arg | Val | His | Val | Val | Leu | Pro |     |
|    |       |                      | 130 |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |
| 10 | gaa   | cgc                  | ggc | gcg | gtc | ggc | gag | ccg | gcc | acc | cct | tcg | acg | gca | ggg | gtg | 480 |
|    | Glu   | Arg                  | Gly | Ala | Val | Gly | Glu | Pro | Ala | Thr | Pro | Ser | Thr | Ala | Gly | Val |     |
|    |       |                      | 145 |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
| 15 | ttt   | ctc                  | aag | tac | cgc | gac | gga | cag | agc | ctc | gac | gcg | ctc | gtg | ccc | gag | 528 |
|    | Phe   | Leu                  | Lys | Tyr | Arg | Asp | Gly | Gln | Ser | Leu | Asp | Ala | Leu | Val | Pro | Glu |     |
|    |       |                      |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
| 20 | atc   | cgc                  | aag | ctg | gtc | acg | cat | gcc | atc | ccg | ggc | ctg | gcc | gag | gac | cgt | 576 |
|    | Ile   | Arg                  | Lys | Leu | Val | Thr | His | Ala | Ile | Pro | Gly | Leu | Ala | Glu | Asp | Arg |     |
|    |       |                      |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |
| 25 | gta   | tcg                  | gtt | gcc | ctg | gtg | gtg | gcc | cag | ccc | gtt | cag | gcc | gca | ccc | gcg | 624 |
|    | Val   | Ser                  | Val | Ala | Leu | Val | Val | Ala | Gln | Pro | Val | Gln | Ala | Ala | Pro | Ala |     |
|    |       |                      | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
| 30 | ccg   | gtc                  | gcg | tgg | cgc | cgc | gtg | ctt | ggc | gta | cag | gtc | gcg | gac | gga | tcg | 672 |
|    | Pro   | Val                  | Ala | Trp | Arg | Arg | Val | Leu | Gly | Val | Gln | Val | Ala | Asp | Gly | Ser |     |
|    |       |                      | 210 |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
| 35 | gta   | tcg                  | gtt | gcc | ctg | gtg | gtg | gcc | cag | ccc | gtt | cag | gcc | gca | ccc | gcg | 720 |
|    | Val   | Ser                  | Val | Ala | Leu | Val | Val | Ala | Gln | Pro | Val | Gln | Ala | Ala | Pro | Ala |     |
|    |       |                      | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
| 40 | ata   | gtg                  | gcg | ggg | gcc | gcg | ctc | tac | gtc | tgg | cgc | acg | cgc | tgg | tcc | cgc | 768 |
|    | Ile   | Val                  | Ala | Gly | Ala | Ala | Leu | Tyr | Val | Trp | Arg | Thr | Arg | Trp | Ser | Arg |     |
|    |       |                      |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |     |
| 45 | ggc   | gaa                  | ggg | cgc | ggc | ggc | gct | ggc | gcc | ggc | gcc | acg | gaa | gga | gcc | ggg | 816 |
|    | Gly   | Glu                  | Gly | Arg | Gly | Gly | Ala | Gly | Ala | Gly | Ala | Thr | Glu | Gly | Ala | Gly |     |
|    |       |                      |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |     |
| 50 | cat   | gac                  | tga |     |     |     |     |     |     |     |     |     |     |     |     |     | 825 |
|    | His   | Asp                  | *   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 55 | <210> | 16                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> | 274                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> | PRT                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> | Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 60 | <400> | 16                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | Met   | Asn                  | Ala | Ile | Gly | Ala | Ile | Gln | Arg | Tyr | Arg | Arg | Gly | Ala | Gly | Trp |     |
|    | 1     |                      |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|    | Ala   | Ala                  | Leu | Val | Leu | Ala | Leu | Ala | Leu | Leu | Ala | Gly | Cys | Gly | Ala | Arg |     |
|    |       |                      |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
|    | Val   | Glu                  | Leu | Leu | Gly | Ala | Ala | Pro | Glu | Asn | Glu | Ala | Asn | Glu | Val | Leu |     |
|    |       |                      | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
|    | Ala   | Ala                  | Leu | Leu | Glu | Ala | Gly | Ile | Ala | Ala | Gln | Lys | Gln | Ser | Gly | Lys |     |
|    |       | 50                   |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |
|    | Ala   | Gly                  | Tyr | Ala | Val | Ser | Val | Pro | Ala | Glu | Ala | Val | Ala | Arg | Ser | Leu |     |
|    | 65    |                      |     |     |     | 70  |     |     |     | 75  |     |     |     |     |     | 80  |     |



[illegible]

30           <210> 17  
              <211> 663  
              <212> DNA  
              <213> Bordetella pertussis

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35      <220>
      <221> CDS
      <222> (1)...(663)

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[illegible]

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|----|---|-----|
|    | gcg ctg ctg tgc gcg ccg cgc ctg cga cgc gcg ata gac ggc gcc gag | 336 |
|    | Ala Leu Leu Cys Ala Pro Arg Leu Arg Arg Ala Ile Asp Gly Ala Glu |     |
| 5  | 100 105 110   |     |
|    | gtc cgt acc ttg cat gcc gcg ctc ggg cgc gat gtg atg aat ttc gcc | 384 |
|    | Val Arg Thr Leu His Ala Ala Leu Gly Arg Asp Val Met Asn Phe Ala |     |
|    | 115 120 125   |     |
| 10 | gtg tct tcc gcg gcg ccg gcc ctg cat gac ggg ctc gcc gcc agt tcg | 432 |
|    | Val Ser Ser Ala Ala Arg Ala Leu His Asp Gly Leu Ala Ala Ser Ser |     |
|    | 130 135 140   |     |
| 15 | gac tgg acc ctg gcc gcc acg gtc cag gcg gcg cag aaa ctg ggc tgg | 480 |
|    | Asp Trp Thr Leu Ala Ala Thr Val Gln Ala Ala Gln Lys Leu Gly Trp |     |
|    | 145 150 155 160   |     |
| 20 | gcc ctg ctg cgc gac gcc gtg cag ggc gcc gcc gac gag ata gcg ctg | 528 |
|    | Ala Leu Leu Arg Asp Ala Val Gln Gly Ala Ala Asp Glu Ile Ala Leu |     |
|    | 165 170 175   |     |
| 25 | cgt tgc gcg ctg aag ttg ccg cgc gac ctt gat ccc gcg ccc gtc ctg | 576 |
|    | Arg Cys Ala Leu Lys Leu Pro Arg Asp Leu Asp Pro Ala Pro Val Leu |     |
|    | 180 185 190   |     |
|    | ccg ccc gag gcg gcg ctt gcg ctg gtg ctg tcc atg ctc gaa atc ctg | 624 |
|    | Pro Pro Glu Ala Ala Leu Ala Leu Val Leu Ser Met Leu Glu Ile Leu |     |
|    | 195 200 205   |     |
| 30 | gat gca gaa tgg ctt tcc tcg ttc ccc gcc caa gcc tga             | 663 |
|    | Asp Ala Glu Trp Leu Ser Ser Phe Pro Ala Gln Ala *               |     |
|    | 210 215 220   |     |
| 35 | <210> 18  |     |
|    | <211> 220   |     |
|    | <212> PRT   |     |
|    | <213> Bordetella pertussis                                      |     |
| 40 | <400> 18  |     |
|    | Met Thr Glu Ala Ser Val Leu Leu Ser Glu Arg Leu Met Ile Phe Asn |     |
|    | 1 5 10 15   |     |
|    | Leu Leu Pro Ser Leu Thr Leu His Ala Ser Arg His Asp Glu Met Phe |     |
|    | 20 25 30  |     |
| 45 | Pro Ala Asp Trp Val Arg Ala Leu Cys Asn Ala Asp Ala Ala Leu Ala |     |
|    | 35 40 45  |     |
|    | Asn Ala Trp His Arg His Trp Ser Arg Trp Ile Leu Cys Glu Leu Gly |     |
|    | 50 55 60  |     |
| 50 | Leu Leu Asn Gln Pro Val Leu Ser Leu Asp Pro Pro Gln Leu Lys Val |     |
|    | 65 70 75 80   |     |
|    | Ala Leu Leu Ser Thr Asp Ala Leu Arg Thr Cys Ala Ala His Ala Gly |     |
|    | 85 90 95  |     |
|    | Ala Leu Leu Cys Ala Pro Arg Leu Arg Arg Ala Ile Asp Gly Ala Glu |     |
|    | 100 105 110   |     |
| 55 | Val Arg Thr Leu His Ala Ala Leu Gly Arg Asp Val Met Asn Phe Ala |     |
|    | 115 120 125   |     |
|    | Val Ser Ser Ala Ala Arg Ala Leu His Asp Gly Leu Ala Ala Ser Ser |     |
|    | 130 135 140   |     |
| 60 | Asp Trp Thr Leu Ala Ala Thr Val Gln Ala Ala Gln Lys Leu Gly Trp |     |
|    | 145 150 155 160   |     |



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|    |   |     |
|----|---|-----|
|    | <400> 21  |     |
|    | atg cgt cag tac cac tac atc acg gag atg atg cgg gtg gcc ctg cag | 48  |
|    | Met Arg Gln Tyr His Tyr Ile Thr Glu Met Met Arg Val Ala Leu Gln |     |
| 5  | 1 5 10 15   |     |
|    | gat ctg tcc acg ctg cgg ata aag ggc cgg gtg gtg caa gtg gtg gga | 96  |
|    | Asp Leu Ser Thr Leu Arg Ile Lys Gly Arg Val Val Gln Val Val Gly |     |
|    | 20 25 30  |     |
| 10 | acg atc atc aag gcc gtc gtt ccg atg gtc aag atc ggc gaa gtg tgc | 144 |
|    | Thr Ile Ile Lys Ala Val Val Pro Met Val Lys Ile Gly Glu Val Cys |     |
|    | 35 40 45  |     |
| 15 | ctg ctg cgc aat ccc ggc gag gac ttc gag atg cac ggc gaa gtg gtg | 192 |
|    | Leu Leu Arg Asn Pro Gly Glu Asp Phe Glu Met His Gly Glu Val Val |     |
|    | 50 55 60  |     |
| 20 | ggc ttt gtc cgc gac gcc gcc ttg ctc acg cct atc ggc gac atg tac | 240 |
|    | Gly Phe Val Arg Asp Ala Ala Leu Leu Thr Pro Ile Gly Asp Met Tyr |     |
|    | 65 70 75 80   |     |
|    | ggg att tcc tcg gcg acc gag gtg ata ccg acc gga cgc acg cat atg | 288 |
|    | Gly Ile Ser Ser Ala Thr Glu Val Ile Pro Thr Gly Arg Thr His Met |     |
|    | 85 90 95  |     |
| 25 | gtc ccc gtc ggt ccg gcc ttg ctg gga cgc gtg ctg gac ggc ctg gga | 336 |
|    | Val Pro Val Gly Pro Gly Leu Leu Gly Arg Val Leu Asp Gly Leu Gly |     |
|    | 100 105 110   |     |
| 30 | cgt ccg ctg gac gcc gcc gag tca ggg ccg ctg cat gcc cac aag ttc | 384 |
|    | Arg Pro Leu Asp Ala Ala Glu Ser Gly Pro Leu His Ala His Lys Phe |     |
|    | 115 120 125   |     |
| 35 | tat ccg gtc ttc gcc gat gcg cca gac ccg ctg acg cgt cgc atc atc | 432 |
|    | Tyr Pro Val Phe Ala Asp Ala Pro Asp Pro Leu Thr Arg Arg Ile Ile |     |
|    | 130 135 140   |     |
| 40 | cat gct ccg ctg gag ctg ggg gtg cgc gta ctg gac ggt ttg ctt aca | 480 |
|    | His Ala Pro Leu Glu Leu Gly Val Arg Val Leu Asp Gly Leu Leu Thr |     |
|    | 145 150 155 160   |     |
|    | tgc ggg gaa ggc cag cgt ctg gga att ttc gca gcc gcc ggc ggc ggc | 528 |
|    | Cys Gly Glu Gly Gln Arg Leu Gly Ile Phe Ala Ala Ala Gly Gly Gly |     |
|    | 165 170 175   |     |
| 45 | aag tcg acc ctg ctg ggc atg ctg gtc aag ggc gcc gcg gtc gac gtg | 576 |
|    | Lys Ser Thr Leu Leu Gly Met Leu Val Lys Gly Ala Ala Val Asp Val |     |
|    | 180 185 190   |     |
| 50 | acg gtg gtg gcg ctg atc gcc gag cgt ggg cgg gaa gtt cgc gag ttc | 624 |
|    | Thr Val Val Ala Leu Ile Gly Glu Arg Gly Arg Glu Val Arg Glu Phe |     |
|    | 195 200 205   |     |
| 55 | ctt gag cac gaa ctc ggt ccg gag ggc aga cgc aag agc gtg atc gtc | 672 |
|    | Leu Glu His Glu Leu Gly Pro Glu Gly Arg Arg Lys Ser Val Ile Val |     |
|    | 210 215 220   |     |
| 60 | tgc gcg acc agc gac aag tcc tcg atg gag cgt gcc aag gcg gcg tac | 720 |
|    | Cys Ala Thr Ser Asp Lys Ser Ser Met Glu Arg Ala Lys Ala Ala Tyr |     |
|    | 225 230 235 240   |     |

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|    |   |      |
|----|---|------|
| 5  | gtc gca acc gcc atc gcc gaa tac ttc cgc gat caa ggg cag cgt gta<br>Val Ala Thr Ala Ile Ala Glu Tyr Phe Arg Asp Gln Gly Gln Arg Val<br>245 250 255     | 768  |
| 10 | ctt ttt ctg atg gac tcg gtc acc cgc ttt gcg cga gcc cag cgt gaa<br>Leu Phe Leu Met Asp Ser Val Thr Arg Phe Ala Arg Ala Gln Arg Glu<br>260 265 270     | 816  |
| 15 | atc ggc ttg gcg gca ggc gag ccg ccg acg cgg cgc ggc tat cca ccg<br>Ile Gly Leu Ala Ala Gly Glu Pro Pro Thr Arg Arg Gly Tyr Pro Pro<br>275 280 285     | 864  |
| 20 | tcg gtg ttc gcc acc ttg ccc aaa ctg atg gag cgc gcc ggc atg aac<br>Ser Val Phe Ala Thr Leu Pro Lys Leu Met Glu Arg Ala Gly Met Asn<br>290 295 300     | 912  |
| 25 | cag acg ggt tcg atc acg gcg ctg tat acg gtg ctg gtc gag ggg gac<br>Gln Thr Gly Ser Ile Thr Ala Leu Tyr Thr Val Leu Val Glu Gly Asp<br>305 310 315 320 | 960  |
| 30 | gac atg aac gaa ccg gtg gcc gac gag acg cgt tcg ata ctg gac ggc<br>Asp Met Asn Glu Pro Val Ala Asp Glu Thr Arg Ser Ile Leu Asp Gly<br>325 330 335     | 1008 |
| 35 | cac atc gtg ctc tcg cgc aag ctg gga gcg gcg aat cac tat cct gcc<br>His Ile Val Leu Ser Arg Lys Leu Gly Ala Ala Asn His Tyr Pro Ala<br>340 345 350     | 1056 |
| 40 | gtc gac gtg ctg gcc tcg gcc agc cgg gtc atg aat gcc gtg gtg tcg<br>Val Asp Val Leu Ala Ser Ala Ser Arg Val Met Asn Ala Val Val Ser<br>355 360 365     | 1104 |
| 45 | ccg cgt cac aag tac ctg gcc gga cgt atg cgc gaa ctg atg gcc aag<br>Pro Arg His Lys Tyr Leu Ala Gly Arg Met Arg Glu Leu Met ala Lys<br>370 375 380     | 1152 |
| 50 | tac cag gat gtc gag ctg ttg gtg aaa atc ggc gag tac aag cag ggc<br>Tyr Gln Asp Val Glu Leu Leu Val Lys Ile Gly Glu Tyr Lys Gln Gly<br>385 390 395 400 | 1200 |
| 55 | gcc gat gcg tcg acc gat gag gcg ata cag aag atc gga cag atc aat<br>Ala Asp Ala Ser Thr Asp Glu Ala Ile Gln Lys Ile Gly Gln Ile Asn<br>405 410 415     | 1248 |
| 60 | gcg ttt ctc aga caa cta acc gac gaa cgc gaa gca ttc gag gat acc<br>Ala Phe Leu Arg Gln Leu Thr Asp Glu Arg Glu Ala Phe Glu Asp Thr<br>420 425 430     | 1296 |
| 65 | gta ctg cgc atg gct gaa atc atc gga ccc gaa tcc taa<br>Val Leu Arg Met ala Glu Ile Ile Gly Pro Glu Ser *<br>435 440                                   | 1335 |
| 70 | <210> 22<br><211> 444<br><212> PRT<br><213> Bordetella pertussis  |      |
| 75 | <400> 22  |      |

25

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    <220>
    <221> CDS
    <222> (1)...(510)

    <400> 23
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    Met Asp Leu Glu Ser Leu Leu Ala Ile Lys His Phe Arg Ala Asp Gln
    1          5          10          15

    gcc cag ctt gcg ctg aaa cgc caa cag cag gcc tgc gcg gtt gct gcc      96
    Ala Gln Leu Ala Leu Lys Arg Gln Gln Gln Ala Cys Ala Val Ala Ala
    20          25          30

    gcg gcg cag cgt cag gcg caa ggc cgc ctc gac gat tgt cgc ctg tgg      144
    Ala Ala Gln Arg Gln Ala Gln Gly Arg Leu Asp Asp Cys Arg Leu Trp
    35          40          45

    gcc gga cag ctc gaa aac cgt cta tat gcc gag ctg tgc cgg cgc atc      192
    Ala Gly Gln Leu Glu Asn Arg Leu Tyr Ala Glu Leu Cys Arg Arg Ile
    50          55          60

    gtc aag aca cgc gac atc gac gag gtg ctg caa cga gtg ggc cac gcc      240
    Val Lys Thr Arg Asp Ile Asp Glu Val Leu Gln Arg Val Gly His Ala
    65          70          75          80

    cgc gac cgc cag gcc agc ctg gcg ctg cag ctc gac gac gcc gtg cgc      288
    Arg Asp Arg Gln Ala Ser Leu Ala Leu Gln Leu Asp Asp Ala Val Arg
    85          90          95

    cgt cac gaa cat gaa atc cag ctg ctc gcg cag cag cgc gag cag cac      336
    Arg His Glu His Glu Ile Gln Leu Leu Ala Gln Gln Arg Glu Gln His
    100          105          110

    cgg gag tgc ttc cag gcg cag caa cgg atc gcc gag ttg gtg cgc ctg      384
    Arg Glu Cys Phe Gln Ala Gln Gln Arg Ile Ala Glu Leu Val Arg Leu
    115          120          125

    cag cag gtc gag gcg gcg gcc ttg cgc gag agc cag gaa gat cgc gaa      432
    Gln Gln Val Glu Ala Ala Ala Leu Arg Glu Ser Gln Glu Asp Arg Glu
    130          135          140

    att cag gaa gcc atc gaa ttg tcg gcg cgt ggg cgc gac gat gca tcg      480
    Ile Gln Glu Ala Ile Glu Leu Ser Ala Arg Gly Arg Asp Asp Ala Ser
    145          150          155          160

    cga gcc ggc gac ggc ctg gcg cgg cta tga      510
    Arg Ala Gly Asp Gly Leu Ala Arg Leu *
    165

    <210> 24
    <211> 169
    <212> PRT
    <213> Bordetella pertussis

    <400> 24
    Met Asp Leu Glu Ser Leu Leu Ala Ile Lys His Phe Arg Ala Asp Gln
    1          5          10          15
  
```





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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Pro                        | Asp | Thr | Thr | Leu | Ser | Val | Arg | Glu | Asp | Gly | Gly | Trp | Ile | Val | Val |     |
|    |                            |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
| 5  | gct                        | ttc | gca | tgc | cga | caa | cgg | gac | gct | tgc | gag | cgc | ctg | cac | gcg | tgc | 432 |
|    | Ala                        | Phe | Ala | Cys | Arg | Gln | Arg | Asp | Ala | Cys | Glu | Arg | Leu | His | Ala | Cys |     |
|    |                            | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |
| 10 | gcc                        | gac | cgg | ttg | gcc | atg | gag | ctc | gcg | ctg | gag | ctg | gag | cgc | gac | gtc | 480 |
|    | Ala                        | Asp | Arg | Leu | Ala | Met | Glu | Leu | Ala | Leu | Glu | Leu | Ala | Arg | Asp | Val |     |
|    |                            | 145 |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
| 15 | gag                        | gtt | gag | gtg | gca | tgc | gac | ggc | gag | ccg | cac | gag | cgg | gtg | gag | cgc | 528 |
|    | Glu                        | Val | Ala | Val | Ala | Cys | Asp | Gly | Glu | Pro | His | Glu | Arg | Val | Ala | Arg |     |
|    |                            |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
| 20 | gcg                        | cag | cgg | ccg | tgg | cga | tga |     |     |     |     |     |     |     |     |     | 549 |
|    | Ala                        | Gln | Arg | Pro | Trp | Arg | *   |     |     |     |     |     |     |     |     |     |     |
|    |                            |     |     |     | 180 |     |     |     |     |     |     |     |     |     |     |     |     |
| 25 | <210> 26                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 182                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> PRT                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 30 | <400> 26                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | Met                        | Asn | Gln | Pro | Asp | Gly | Leu | Gly | Ser | Pro | Met | ala | Gly | Gly | Gly | Gln |     |
|    | 1                          |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|    | Arg                        | Met | Gly | Val | Ala | Arg | Thr | Pro | Tyr | Ala | Arg | Gln | Pro | Asp | Arg | Asp |     |
|    |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
|    | Ala                        | Gln | Arg | Ala | Phe | Glu | Arg | Glu | Met | Glu | Gln | Glu | Lys | Ala | Lys | Glu |     |
|    |                            |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
|    | Glu                        | Leu | Pro | Gly | Pro | Gln | Arg | Leu | Ala | Pro | Gly | Pro | Ala | Cys | Val | Gly |     |
|    |                            | 50  |     |     |     | 55  |     |     |     |     |     | 60  |     |     |     |     |     |
| 35 | Trp                        | Leu | Ala | Ser | Met | Glu | Pro | Ala | Ala | Gly | Arg | Pro | Pro | Ala | Ser | Leu |     |
|    | 65                         |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
|    | Ala                        | Gln | Ala | Leu | Ala | Ser | Val | Ala | Ala | Gly | Leu | Ala | Val | Gly | Asp | Val |     |
|    |                            |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
| 40 | Leu                        | Glu | Gly | Tyr | Arg | Glu | Ala | Arg | Ile | Val | Val | Asp | Asp | Thr | Leu | Leu |     |
|    |                            |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
|    | Pro                        | Asp | Thr | Thr | Leu | Ser | Val | Arg | Glu | Asp | Gly | Gly | Trp | Ile | Val | Val |     |
|    |                            |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
|    | Ala                        | Phe | Ala | Cys | Arg | Gln | Arg | Asp | Ala | Cys | Glu | Arg | Leu | His | Ala | Cys |     |
|    |                            | 130 |     |     |     | 135 |     |     |     |     |     | 140 |     |     |     |     |     |
| 45 | Ala                        | Asp | Arg | Leu | Ala | Met | Glu | Leu | Ala | Leu | Glu | Leu | Ala | Arg | Asp | Val |     |
|    | 145                        |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
|    | Glu                        | Val | Ala | Val | Ala | Cys | Asp | Gly | Glu | Pro | His | Glu | Arg | Val | Ala | Arg |     |
|    |                            |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
| 50 | Ala                        | Gln | Arg | Pro | Trp | Arg |     |     |     |     |     |     |     |     |     |     |     |
|    |                            |     |     | 180 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 55 | <210> 27                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 1080                 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> DNA                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 60 | <220>                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <221> CDS                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <222> (1)...(1080)         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

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|    |   |      |
|----|---|------|
|    | gga ccc agc gcg atc cgg gta cgg gcc gag cat gcg tct ttt cgt gta | 768  |
|    | Gly Pro Ser Ala Ile Arg Val Arg Ala Glu His Ala Ser Phe Arg Val |      |
|    | 245 250 255   |      |
| 5  | act caa ggt tgg act ccc atc atg acg gaa ccc gcg aca cct gac cct | 816  |
|    | Thr Gln Gly Trp Thr Pro Ile Met Thr Glu Pro Ala Thr Pro Asp Pro |      |
|    | 260 265 270   |      |
| 10 | ggc gaa acc ccg gca cag gcc gac gcg acg ctc gat acc gat cag ata | 864  |
|    | Gly Glu Thr Pro Ala Gln Ala Asp Ala Thr Leu Asp Thr Asp Gln Ile |      |
|    | 275 280 285   |      |
| 15 | ccc gtg cgc ctg acg ttc gac ctg ggc gag cgc gag ttc acg ctt gcg | 912  |
|    | Pro Val Arg Leu Thr Phe Asp Leu Gly Glu Arg Glu Phe Thr Leu Ala |      |
|    | 290 295 300   |      |
| 20 | cag ctg cgc agc ctg cat ccg ggc tgc acg ttc gac ctc gag cgg ccc | 960  |
|    | Gln Leu Arg Ser Leu His Pro Gly Cys Thr Phe Asp Leu Glu Arg Pro |      |
|    | 305 310 315 320   |      |
|    | atc gcc gac ggg ccg gtc atg gtg cgg gcc aat ggc ctg ttg ctg ggc | 1008 |
|    | Ile Ala Asp Gly Pro Val Met Val Arg Ala Asn Gly Leu Leu Leu Gly |      |
|    | 325 330 335   |      |
| 25 | agc ggc cgg ctg gtc gac atc gac ggc cgc atc ggc gtg gta ttg cag | 1056 |
|    | Ser Gly Arg Leu Val Asp Ile Asp Gly Arg Ile Gly Val Val Leu Gln |      |
|    | 340 345 350   |      |
| 30 | tcg gtc agg cct gga ctc gca tga                                 | 1080 |
|    | Ser Val Arg Pro Gly Leu Ala *                                   |      |
|    | 355   |      |
| 35 | <210> 28  |      |
|    | <211> 359   |      |
|    | <212> PRT   |      |
|    | <213> Bordetella pertussis                                      |      |
| 40 | <400> 28  |      |
|    | Met Asn Arg Val Ala Gly Gly Ala Ala Ala Gln Ala Ala Gly Met Val |      |
|    | 1 5 10 15   |      |
|    | Asp Leu Ala Val Pro Arg Leu Ser Ala Gly Glu Ala His Ala Leu Ser |      |
|    | 20 25 30  |      |
| 45 | Arg Ile Ala Cys His Gly Ala Arg Phe Asp Val Arg Leu Gly Glu Pro |      |
|    | 35 40 45  |      |
|    | Ala Val Arg Trp His Cys Ala Leu Thr Pro Cys Val His Gly Asp Leu |      |
|    | 50 55 60  |      |
| 50 | Ala Asp Gly Glu Met Glu Ser Leu Gln Leu Gln Trp Ala Gly Thr Tyr |      |
|    | 65 70 75 80   |      |
|    | Ile Gly Leu Thr Val Pro Arg Ala Ala Ala Gly Trp Leu Ala Ala     |      |
|    | 85 90 95  |      |
|    | Arg Leu Pro Arg Phe Ser Gly Val Glu Leu Pro Glu Pro Ile Ala Ala |      |
|    | 100 105 110   |      |
| 55 | Ala Ala Leu Glu Ala Met Leu Glu Glu Val Cys Arg Gly Val Ala Gly |      |
|    | 115 120 125   |      |
|    | Leu Asp Gln Gln Gly Pro Val Arg Val Ala Arg Gln Gly Gly Thr Pro |      |
|    | 130 135 140   |      |
| 60 | Pro Val Gln Pro His Arg Trp Thr Leu Thr Val Arg Ala Pro Asp Gly |      |
|    | 145 150 155 160   |      |



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5 gcc gtg ctt ggc gtg gcc gag cga ggc gtg ggg ccg ctg cgg gcc ttc 336  
Ala Val Leu Gly Val Ala Glu Arg Gly Val Gly Pro Leu Arg Ala Phe  
100 105 110

atg ttg cgc aac agc cag ccg gcc cag cgt gat ttc ttc ctg cgc aca 384  
Met Leu Arg Asn Ser Gln Pro Ala Gln Arg Asp Phe Phe Leu Arg Thr  
115 120 125

10 gcg cgt cat ctc tgg ggc gag gag gca tcg cgg gac ctg tcg gaa gac 432  
Ala Arg His Leu Trp Gly Glu Glu Ala Ser Arg Asp Leu Ser Glu Asp  
130 135 140

15 aac ctg ctg gta ttg acg ccc gca ttt ctg gtt tcg gag ctg acc gcc 480  
Asn Leu Leu Val Leu Thr Pro Ala Phe Leu Val Ser Glu Leu Thr Ala  
145 150 155 160

20 gca ttc cag ctt ggc ttt ctg ctg tac ctg ccg ttc atc atc atc gac 528  
Ala Phe Gln Leu Gly Phe Leu Leu Tyr Leu Pro Phe Ile Ile Ile Asp  
165 170 175

25 ctc atc gta tcg aac att ctt ctt gcc atg gga atg atg atg gtt tct 576  
Leu Ile Val Ser Asn Ile Leu Leu Ala Met Gly Met Met Met Val Ser  
180 185 190

ccc gtg acg atc tcc atg ccg ttg aag ctg ttc ctg ttc gtc atg gtg 624  
Pro Val Thr Ile Ser Met Pro Leu Lys Leu Phe Leu Phe Val Met Val  
195 200 205

30 gac ggc tgg acg cgc ctg atc cag ggc ctg gtg ctt tcc tat cgg tga 672  
Asp Gly Trp Thr Arg Leu Ile Gln Gly Leu Val Leu Ser Tyr Arg \*  
210 215 220

35 <210> 30  
<211> 223  
<212> PRT  
<213> Bordetella pertussis

40 <400> 30  
Met Ser Asp Thr Asp Pro Phe Ser Leu Ala Leu Phe Leu Ala Leu Leu  
1 5 10 15  
Ala Leu Val Pro Leu Ile Val Val Met Thr Thr Ser Phe Leu Lys Ile  
20 25 30

45 Ala Val Val Leu Ala Leu Val Arg Asn Ala Leu Gly Val Gln Gln Val  
35 40 45  
Pro Pro Asn Met ala Leu Tyr Gly Leu Ala Leu Ile Leu Ser Ala Tyr  
50 55 60  
Val Met ala Pro Val Val His Arg Ile Gly Thr Glu Val Gln Ala Leu  
65 70 75 80  
Thr Ala Gln Ala Gly Glu Ser Gly Thr Ala Ala Pro Met ala Leu Asp  
85 90 95  
Ala Val Leu Gly Val Ala Glu Arg Gly Val Gly Pro Leu Arg Ala Phe  
100 105 110

55 Met Leu Arg Asn Ser Gln Pro Ala Gln Arg Asp Phe Phe Leu Arg Thr  
115 120 125  
Ala Arg His Leu Trp Gly Glu Glu Ala Ser Arg Asp Leu Ser Glu Asp  
130 135 140  
Asn Leu Leu Val Leu Thr Pro Ala Phe Leu Val Ser Glu Leu Thr Ala  
145 150 155 160

60

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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Ala                        | Phe | Gln | Leu | Gly | Phe | Leu | Leu | Tyr | Leu | Pro | Phe | Ile | Ile | Ile | Asp |     |
|    |                            |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
|    | Leu                        | Ile | Val | Ser | Asn | Ile | Leu | Leu | Ala | Met | Gly | Met | Met | Met | Val | Ser |     |
|    |                            |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |
| 5  | Pro                        | Val | Thr | Ile | Ser | Met | Pro | Leu | Lys | Leu | Phe | Leu | Phe | Val | Met | Val |     |
|    |                            |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
|    | Asp                        | Gly | Trp | Thr | Arg | Leu | Ile | Gln | Gly | Leu | Val | Leu | Ser | Tyr | Arg |     |     |
|    |                            | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
| 10 | <210> 31                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 267                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> DNA                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 15 | <220>                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <221> CDS                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <222> (1)...(267)          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <400> 31                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 20 | atg                        | caa | acc | caa | gac | ctg | gtt | tcg | ttc | atg | aca | cag | gcg | ttg | tac | ctg | 48  |
|    | Met                        | Gln | Thr | Gln | Asp | Leu | Val | Ser | Phe | Met | Thr | Gln | Ala | Leu | Tyr | Leu |     |
|    | 1                          |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
| 25 | gtg                        | ctc | tgg | ctg | tcg | ctg | ccg | ccc | atc | gcc | gtg | gtg | gcg | atc | gtg | gga | 96  |
|    | Val                        | Leu | Trp | Leu | Ser | Leu | Pro | Pro | Ile | Ala | Val | Val | Ala | Ile | Val | Gly |     |
|    |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| 30 | acg                        | ctg | ttt | tcc | ctg | ttg | cag | gcc | ttg | acg | cag | gtg | cag | gag | cag | acc | 144 |
|    | Thr                        | Leu | Phe | Ser | Leu | Leu | Gln | Ala | Leu | Thr | Gln | Val | Gln | Glu | Gln | Thr |     |
|    |                            |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
| 35 | ctg                        | tcc | ttc | gcc | gtg | aag | ctg | ata | gcc | gtg | ttc | gcc | acg | ctg | atg | ctg | 192 |
|    | Leu                        | Ser | Phe | Ala | Val | Lys | Leu | Ile | Ala | Val | Phe | Ala | Thr | Leu | Met | Leu |     |
|    |                            | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |     |
| 40 | gcg                        | gcc | ccg | tgg | ata | agc | gcg | gaa | atc | tat | aac | ttc | acg | att | gcg | gtg | 240 |
|    | Ala                        | Ala | Arg | Trp | Ile | Ser | Ala | Glu | Ile | Tyr | Asn | Phe | Thr | Ile | Ala | Val |     |
|    | 65                         |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     | 80  |     |     |
| 45 | ttc                        | gat | gcc | ttt | cat | cgg | atc | cac | tga |     |     |     |     |     |     |     | 267 |
|    | Phe                        | Asp | Ala | Phe | His | Arg | Ile | His | *   |     |     |     |     |     |     |     |     |
|    |                            |     |     |     | 85  |     |     |     |     |     |     |     |     |     |     |     |     |
| 50 | <210> 32                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 88                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> PRT                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 55 | <400> 32                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | Met                        | Gln | Thr | Gln | Asp | Leu | Val | Ser | Phe | Met | Thr | Gln | Ala | Leu | Tyr | Leu |     |
|    | 1                          |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|    | Val                        | Leu | Trp | Leu | Ser | Leu | Pro | Pro | Ile | Ala | Val | Val | Ala | Ile | Val | Gly |     |
|    |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| 60 | Thr                        | Leu | Phe | Ser | Leu | Leu | Gln | Ala | Leu | Thr | Gln | Val | Gln | Glu | Gln | Thr |     |
|    |                            |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |     |
|    | Leu                        | Ser | Phe | Ala | Val | Lys | Leu | Ile | Ala | Val | Phe | Ala | Thr | Leu | Met | Leu |     |
|    |                            | 50  |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |     |
|    | Ala                        | Ala | Arg | Trp | Ile | Ser | Ala | Glu | Ile | Tyr | Asn | Phe | Thr | Ile | Ala | Val |     |
|    | 65                         |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     | 80  |     |     |

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Phe Asp Ala Phe His Arg Ile His  
85

5 <210> 33  
<211> 801  
<212> DNA  
<213> Bordetella pertussis

10 <220>  
<221> CDS  
<222> (1)...(801)

<400> 33

15 atg cac acg gag ttc aat ttc gtc gag gcg aag gtt ttc ctg gga acg 48  
Met His Thr Glu Phe Asn Phe Val Glu Ala Lys Val Phe Leu Gly Thr  
1 5 10 15

20 ctg gcc atg acg caa ccg cgg ata ctc acg gcc atg ctc ttt ctg ccg 96  
Leu Ala Met Thr Gln Pro Arg Ile Leu Thr Ala Met Leu Phe Leu Pro  
20 25 30

25 atg ttc aac cgt cag ttt ctg cct ggt ccg ctg cgt tac gcc gtc ggc 144  
Met Phe Asn Arg Gln Phe Leu Pro Gly Pro Leu Arg Tyr Ala Val Gly  
35 40 45

30 gcc tgt ctc ggg ctg atc gtg gtt ccc cag ctg gcg ccg cag tat gcc 192  
Ala Cys Leu Gly Leu Ile Val Val Pro Gln Leu Ala Pro Gln Tyr Ala  
50 55 60

35 gcg ctg gat atc gac tgg ccc cgg ctg ctg gcg ctg ctg gcc aag gag 240  
Ala Leu Asp Ile Asp Trp Pro Arg Leu Leu Ala Leu Leu Ala Lys Glu  
65 70 75 80

40 gcg atg gtg ggc atg ttc ctg ggt tgg ctg gct gcc ttg cca ttc tgg 288  
Ala Met Val Gly Met Phe Leu Gly Trp Leu Ala Ala Leu Pro Phe Trp  
85 90 95

45 atc ttc gag gcc atc ggc ttc gtc ata gac aac caa cgg ggc gcc agc 336  
Ile Phe Glu Ala Ile Gly Phe Val Ile Asp Asn Gln Arg Gly Ala Ser  
100 105 110

50 ctg ggc gct atc ctc aac ccc gcc acg ggc aac gat tgc tgc ccc atg 384  
Leu Gly Ala Ile Leu Asn Pro Ala Thr Gly Asn Asp Ser Ser Pro Met  
115 120 125

55 ggc att ctc ttc aat ctg gga ttc atg gtg ttc ttc ctg acg gcg ggc 432  
Gly Ile Leu Phe Asn Leu Gly Phe Met Val Phe Phe Leu Thr Ala Gly  
130 135 140

60 gga ttc ggg ttg ttc gcc acg atg ctg tat gac agc ttc ggg ttg tgg 480  
Gly Phe Gly Leu Phe Ala Thr Met Leu Tyr Asp Ser Phe Gly Leu Trp  
145 150 155 160

55 aac atc tgg gcg tgg tgg ccg tcc atg ccc gca cag ggc gcc gtg cgg 528  
Asn Ile Trp Ala Trp Trp Pro Ser Met Pro Ala Gln Gly Ala Val Arg  
165 170 175

60 atg ctg gac cag ttc agt ggc ttt gcc gcg cgt gtc ctg ctg ctg gcc 576  
Met Leu Asp Gln Phe Ser Gly Phe Ala Ala Arg Val Leu Leu Leu Ala  
180 185 190



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|    |   |     |
|----|---|-----|
|    | tcg ccg gcc atc gtg gcc atg ttc ctg gcc gag ctg ggc ctg gcc ctg | 624 |
|    | Ser Pro Ala Ile Val Ala Met Phe Leu Ala Glu Leu Gly Leu Ala Leu |     |
|    | 195 200 205   |     |
| 5  | atc agc cgc ttc gcg cct caa ctg cag gtg ttc ttc ctg gct ctg ccg | 672 |
|    | Ile Ser Arg Phe Ala Pro Gln Leu Gln Val Phe Phe Leu Ala Leu Pro |     |
|    | 210 215 220   |     |
| 10 | gta aag agc gcg ctg gtg ctg ttc gtg ctg gtg ctg tac atg gca acg | 720 |
|    | Val Lys Ser Ala Leu Val Leu Phe Val Leu Val Leu Tyr Met ala Thr |     |
|    | 225 230 235 240   |     |
| 15 | ttg ttc cag tat gca ggc gaa atc ctg ggt tct gtg ggc cgg atc gtg | 768 |
|    | Leu Phe Gln Tyr Ala Gly Glu Ile Leu Gly Ser Val Gly Arg Ile Val |     |
|    | 245 250 255   |     |
| 20 | ccg ttc ctg cat tca gcg tgg ccc ggc cca tga                     | 801 |
|    | Pro Phe Leu His Ser Ala Trp Pro Gly Pro *                       |     |
|    | 260 265   |     |
| 25 | <210> 34  |     |
|    | <211> 266   |     |
|    | <212> PRT   |     |
|    | <213> Bordetella pertussis                                      |     |
| 30 | <400> 34  |     |
|    | Met His Thr Glu Phe Asn Phe Val Glu Ala Lys Val Phe Leu Gly Thr |     |
|    | 1 5 10 15   |     |
|    | Leu Ala Met Thr Gln Pro Arg Ile Leu Thr Ala Met Leu Phe Leu Pro |     |
|    | 20 25 30  |     |
|    | Met Phe Asn Arg Gln Phe Leu Pro Gly Pro Leu Arg Tyr Ala Val Gly |     |
|    | 35 40 45  |     |
| 35 | Ala Cys Leu Gly Leu Ile Val Val Pro Gln Leu Ala Pro Gln Tyr Ala |     |
|    | 50 55 60  |     |
|    | Ala Leu Asp Ile Asp Trp Pro Arg Leu Leu Ala Leu Ala Lys Glu     |     |
|    | 65 70 75 80   |     |
| 40 | Ala Met Val Gly Met Phe Leu Gly Trp Leu Ala Ala Leu Pro Phe Trp |     |
|    | 85 90 95  |     |
|    | Ile Phe Glu Ala Ile Gly Phe Val Ile Asp Asn Gln Arg Gly Ala Ser |     |
|    | 100 105 110   |     |
|    | Leu Gly Ala Ile Leu Asn Pro Ala Thr Gly Asn Asp Ser Ser Pro Met |     |
|    | 115 120 125   |     |
| 45 | Gly Ile Leu Phe Asn Leu Gly Phe Met Val Phe Phe Leu Thr Ala Gly |     |
|    | 130 135 140   |     |
|    | Gly Phe Gly Leu Phe Ala Thr Met Leu Tyr Asp Ser Phe Gly Leu Trp |     |
|    | 145 150 155 160   |     |
| 50 | Asn Ile Trp Ala Trp Trp Pro Ser Met Pro Ala Gln Gly Ala Val Arg |     |
|    | 165 170 175   |     |
|    | Met Leu Asp Gln Phe Ser Gly Phe Ala Ala Arg Val Leu Leu Leu Ala |     |
|    | 180 185 190   |     |
|    | Ser Pro Ala Ile Val Ala Met Phe Leu Ala Glu Leu Gly Leu Ala Leu |     |
|    | 195 200 205   |     |
| 55 | Ile Ser Arg Phe Ala Pro Gln Leu Gln Val Phe Phe Leu Ala Leu Pro |     |
|    | 210 215 220   |     |
|    | Val Lys Ser Ala Leu Val Leu Phe Val Leu Val Leu Tyr Met ala Thr |     |
|    | 225 230 235 240   |     |
| 60 | Leu Phe Gln Tyr Ala Gly Glu Ile Leu Gly Ser Val Gly Arg Ile Val |     |
|    | 245 250 255   |     |



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|    |   |      |
|----|---|------|
|    | ctg gcg tac ggg gcg att tcg ctg gcg gac ctg gcc tgg cag cgt tac | 624  |
|    | Leu Ala Tyr Gly Ala Ile Ser Leu Ala Asp Leu Ala Trp Gln Arg Tyr |      |
| 5  | 195 200 205   |      |
|    | cag tat cgc aaa ggc ttg cgg atg agc aag gac gaa gtg aag cag gag | 672  |
|    | Gln Tyr Arg Lys Gly Leu Arg Met Ser Lys Asp Glu Val Lys Gln Glu |      |
|    | 210 215 220   |      |
| 10 | tac aag gag atg gaa ggc gat ccc cat atc aag cag caa cgc aag cac | 720  |
|    | Tyr Lys Glu Met Glu Gly Asp Pro His Ile Lys Gln Gln Arg Lys His |      |
|    | 225 230 235 240   |      |
| 15 | ctg cac cag gag ctg atc atg cat ggc gcg gcg gcc cag gtt cgc cgg | 768  |
|    | Leu His Gln Glu Leu Ile Met His Gly Ala Ala Ala Gln Val Arg Arg |      |
|    | 245 250 255   |      |
| 20 | gcg acg gtg ctg gtg acc aat ccg aca cac ctg gcc gtg gcc ctg tac | 816  |
|    | Ala Thr Val Leu Val Thr Asn Pro Thr His Leu Ala Val Ala Leu Tyr |      |
|    | 260 265 270   |      |
|    | tac gcg gcg ggc gag acg ccc ttg ccg cgc gtg ctg gcc atg ggg cag | 864  |
|    | Tyr Ala Ala Gly Glu Thr Pro Leu Pro Arg Val Leu Ala Met Gly Gln |      |
|    | 275 280 285   |      |
| 25 | gga gcc gtg gcc gct ctc atg gtc gag gcc gcg cgc gat gcc ggc gtg | 912  |
|    | Gly Ala Val Ala Ala Leu Met Val Glu Ala Ala Arg Asp Ala Gly Val |      |
|    | 290 295 300   |      |
| 30 | ccg gtc atg cag aac gtc gcg ctg gcc cgc gcc ttg cac gac cag gcg | 960  |
|    | Pro Val Met Gln Asn Val Ala Leu Ala Arg Ala Leu His Asp Gln Ala |      |
|    | 305 310 315 320   |      |
| 35 | gag gtg gac caa tac att ccc ggc gag ttg gtg gag ccg gtg gcc gcg | 1008 |
|    | Glu Val Asp Gln Tyr Ile Pro Gly Glu Leu Val Glu Pro Val Ala Ala |      |
|    | 325 330 335   |      |
| 40 | gtg ttg cgg gcg gtg cgc cag gca ctc aag gag cag aca tga         | 1050 |
|    | Val Leu Arg Ala Val Arg Gln Ala Leu Lys Glu Gln Thr *           |      |
|    | 340 345   |      |
| 45 | <210> 36  |      |
|    | <211> 349   |      |
|    | <212> PRT   |      |
|    | <213> Bordetella pertussis                                      |      |
| 50 | <400> 36  |      |
|    | Met Ser Gly Glu Lys Thr Glu Arg Pro Thr Pro Lys Arg Leu Arg Asp |      |
|    | 1 5 10 15   |      |
|    | Ser Arg Glu Lys Gly Glu Val Ala His Ser Arg Asp Phe Thr Gln Thr |      |
|    | 20 25 30  |      |
|    | Ala Leu Ile Cys Ala Leu Phe Gly His Phe Leu Ile Asn Ala Pro Ser |      |
|    | 35 40 45  |      |
| 55 | Ile Leu Ala Ser Leu Arg Ala Leu Ile Leu Ala Pro Ala Ala Phe Ala |      |
|    | 50 55 60  |      |
|    | Asp Gln Gly Phe Ala Val Ala Leu Gly Pro Val Leu Thr Glu Ile Leu |      |
|    | 65 70 75 80   |      |
| 60 | Asp Gln Ala Val Arg Val Leu Ala Pro Leu Ile Leu Ile Val Leu Gly |      |
|    | 85 90 95  |      |

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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Val                        | Gly | Met | Phe | Ala | Glu | Phe | Leu | Gln | Val | Gly | Val | Val | Leu | Ala | Phe |     |
|    |                            |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
|    | Arg                        | Lys | Leu | Lys | Pro | Ser | Ala | Glu | Lys | Leu | Asn | Pro | Ala | Gly | Asn | Leu |     |
|    |                            |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
| 5  | Lys                        | Asn | Ile | Phe | Ser | Ala | Arg | Asn | Leu | Met | Glu | Phe | Ile | Lys | Ser | Val |     |
|    |                            |     | 130 |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |
|    | Cys                        | Lys | Ile | Leu | Phe | Leu | Ala | Val | Leu | Val | Thr | Leu | Val | Ile | Arg | Asp |     |
|    |                            |     | 145 |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
| 10 | Ser                        | Leu | Gln | Pro | Leu | Met | ala | Val | Pro | His | Ser | Gly | Leu | Asp | Gly | Leu |     |
|    |                            |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     |     | 175 |     |
|    | Arg                        | Thr | Gly | Val | Gly | Arg | Ile | Leu | Gln | Val | Met | Val | Trp | Asn | Ile | Gly |     |
|    |                            |     |     | 180 |     |     |     |     | 185 |     |     |     |     |     | 190 |     |     |
|    | Leu                        | Ala | Tyr | Gly | Ala | Ile | Ser | Leu | Ala | Asp | Leu | Ala | Trp | Gln | Arg | Tyr |     |
|    |                            |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
| 15 | Gln                        | Tyr | Arg | Lys | Gly | Leu | Arg | Met | Ser | Lys | Asp | Glu | Val | Lys | Gln | Glu |     |
|    |                            |     |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
|    | Tyr                        | Lys | Glu | Met | Glu | Gly | Asp | Pro | His | Ile | Lys | Gln | Gln | Arg | Lys | His |     |
|    |                            |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |
| 20 | Leu                        | His | Gln | Glu | Leu | Ile | Met | His | Gly | Ala | Ala | Ala | Gln | Val | Arg | Arg |     |
|    |                            |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     |     | 255 |     |
|    | Ala                        | Thr | Val | Leu | Val | Thr | Asn | Pro | Thr | His | Leu | Ala | Val | Ala | Leu | Tyr |     |
|    |                            |     |     | 260 |     |     |     |     | 265 |     |     |     |     |     | 270 |     |     |
|    | Tyr                        | Ala | Ala | Gly | Glu | Thr | Pro | Leu | Pro | Arg | Val | Leu | Ala | Met | Gly | Gln |     |
|    |                            |     |     | 275 |     |     |     | 280 |     |     |     |     | 285 |     |     |     |     |
| 25 | Gly                        | Ala | Val | Ala | Ala | Leu | Met | Val | Glu | Ala | Ala | Arg | Asp | Ala | Gly | Val |     |
|    |                            |     |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |     |
|    | Pro                        | Val | Met | Gln | Asn | Val | Ala | Leu | Ala | Arg | Ala | Leu | His | Asp | Gln | Ala |     |
|    |                            |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |     |
| 30 | Glu                        | Val | Asp | Gln | Tyr | Ile | Pro | Gly | Glu | Leu | Val | Glu | Pro | Val | Ala | Ala |     |
|    |                            |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |     |
|    | Val                        | Leu | Arg | Ala | Val | Arg | Gln | Ala | Leu | Lys | Glu | Gln | Thr |     |     |     |     |
|    |                            |     |     | 340 |     |     |     |     | 345 |     |     |     |     |     |     |     |     |
| 35 | <210> 37                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 399                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> DNA                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 40 | <220>                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <221> CDS                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <222> (1)... (399)         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <400> 37                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 45 | atg                        | aca | gca | acc | att | cat | ccc | gat | att | gcc | gat | tat | gcg | cga | cgc | cat | 48  |
|    | Met                        | Thr | Ala | Thr | Ile | His | Pro | Asp | Ile | Ala | Asp | Tyr | Ala | Arg | Arg | His |     |
|    | 1                          |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
| 50 | ggc                        | ctc | gaa | ccc | tcg | gtc | gac | gcc | gat | ggc | ggg | ctt | gcc | gtc | cgg | atc | 96  |
|    | Gly                        | Leu | Glu | Pro | Ser | Val | Asp | Ala | Asp | Gly | Gly | Leu | Ala | Val | Arg | Ile |     |
|    |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| 55 | gac                        | gga | cgg | cat | cgc | gtc | agg | ttg | atc | ccc | gcc | gaa | gac | ggc | atg | ctg | 144 |
|    | Asp                        | Gly | Arg | His | Arg | Val | Arg | Leu | Ile | Pro | Ala | Glu | Asp | Gly | Met | Leu |     |
|    |                            |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
|    | gtg                        | ttg | cgg | gcg | cgg | ctg | gcc | gag | ctg | ccc | gat | ggg | tgg | cag | gcg | cgc | 192 |
|    | Val                        | Leu | Arg | Ala | Arg | Leu | Ala | Glu | Leu | Pro | Asp | Gly | Trp | Gln | Ala | Arg |     |
|    |                            | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |
| 60 | gcg                        | gcg | cag | ttg | cgc | cgg | gcg | ggc | ctg | ctg | gcc | agc | gcc | atg | gcc | cct | 240 |

[illegible]

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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Asp                        | Asn | Ala | Tyr | Pro | Asp | Ile | Ala | Thr | Glu | Arg | Ser | Asp | Gln | Gln | Leu |     |
|    |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| 5  | ctg                        | agc | agc | ctg | gta | gcc | gaa | cat | gcc | ggc | cga | tta | cag | aga | ttc | atc | 144 |
|    | Leu                        | Ser | Ser | Leu | Val | Ala | Glu | His | Ala | Gly | Arg | Leu | Gln | Arg | Phe | Ile |     |
|    |                            |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
| 10 | gcc                        | aag | cac | atc | ggc | cac | agc | agc | gac | gtc | gag | gac | ctt | gcg | cag | cag | 192 |
|    | Ala                        | Lys | His | Ile | Gly | His | Ser | Ser | Asp | Val | Glu | Asp | Leu | Ala | Gln | Gln |     |
|    |                            |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| 15 | gct                        | ttc | gcc | gag | gcg | gcg | cgc | gcg | tat | caa | tcg | ttc | cgt | ggc | gac | tcc | 240 |
|    | Ala                        | Phe | Ala | Glu | Ala | Ala | Arg | Ala | Tyr | Gln | Ser | Phe | Arg | Gly | Asp | Ser |     |
|    |                            | 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
| 20 | cag                        | ctt | tcc | acc | tgg | ctg | tac | ggc | atc | gcg | ctc | aat | ctg | gtc | cgc | aat | 288 |
|    | Gln                        | Leu | Ser | Thr | Trp | Leu | Tyr | Gly | Ile | Ala | Leu | Asn | Leu | Val | Arg | Asn |     |
|    |                            |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
| 25 | cac                        | ttg | tcg | cgt | gcg | cca | gag | cgc | cgt | tat | gaa | ttc | acc | tcc | gac | gcc | 336 |
|    | His                        | Leu | Ser | Arg | Ala | Pro | Glu | Arg | Arg | Tyr | Glu | Phe | Thr | Ser | Asp | Ala |     |
|    |                            |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
| 30 | agc                        | ctg | ggt | gtc | atg | cca | tgc | agt | gcg | ccc | aac | ccc | gaa | gcc | gtg | acc | 384 |
|    | Ser                        | Leu | Gly | Val | Met | Pro | Cys | Ser | Ala | Pro | Asn | Pro | Glu | Ala | Val | Thr |     |
|    |                            |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
| 35 | gag                        | cag | cgt | caa | cgc | atg | cgc | ttg | cta | cgc | gaa | gcg | ctg | gag | cag | ctc | 432 |
|    | Glu                        | Gln | Arg | Gln | Arg | Met | Arg | Leu | Leu | Arg | Glu | Ala | Leu | Glu | Gln | Leu |     |
|    |                            |     | 130 |     |     |     | 135 |     |     |     |     |     | 140 |     |     |     |     |
| 40 | ccc                        | gaa | agc | atg | cgc | gac | gtg | atc | ctc | atg | gtc | ggc | gtg | gaa | gaa | ctc | 480 |
|    | Pro                        | Glu | Ser | Met | Arg | Asp | Val | Ile | Leu | Met | Val | Gly | Val | Glu | Glu | Leu |     |
|    |                            |     | 145 |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
| 45 | tcc                        | tat | gaa | gag | gct | gcc | gca | ctg | ctt | tcg | gtt | cct | gta | gga | acc | att | 528 |
|    | Ser                        | Tyr | Glu | Glu | Ala | Ala | Ala | Leu | Leu | Ser | Val | Pro | Val | Gly | Thr | Ile |     |
|    |                            |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
| 50 | cgc                        | agc | cga | ctt | tcc | cgc | gcc | cgc | tgt | gcc | ttg | cgc | gaa | gcg | ctg | cgc | 576 |
|    | Arg                        | Ser | Arg | Leu | Ser | Arg | Ala | Arg | Cys | Ala | Leu | Arg | Glu | Ala | Leu | Arg |     |
|    |                            |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |
| 55 | gaa                        | cga | ggc | tac | gac | agc | gtg | ccg | tag |     |     |     |     |     |     |     | 603 |
|    | Glu                        | Arg | Gly | Tyr | Asp | Ser | Val | Pro | *   |     |     |     |     |     |     |     |     |
|    |                            |     | 195 |     |     |     |     | 200 |     |     |     |     |     |     |     |     |     |
| 60 | <210> 40                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 200                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> PRT                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 65 | <400> 40                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | Met                        | Val | Ser | Pro | Pro | Ser | Ser | Gly | Leu | Pro | Ala | Ser | Leu | Glu | Lys | Pro |     |
|    | 1                          |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |     |     |
|    | Asp                        | Asn | Ala | Tyr | Pro | Asp | Ile | Ala | Thr | Glu | Arg | Ser | Asp | Gln | Gln | Leu |     |
|    |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
|    | Leu                        | Ser | Ser | Leu | Val | Ala | Glu | His | Ala | Gly | Arg | Leu | Gln | Arg | Phe | Ile |     |
|    |                            |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |

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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Ala                        | Lys | His | Ile | Gly | His | Ser | Ser | Asp | Val | Glu | Asp | Leu | Ala | Gln | Gln |     |
|    | 50                         |     |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |     |
|    | Ala                        | Phe | Ala | Glu | Ala | Ala | Arg | Ala | Tyr | Gln | Ser | Phe | Arg | Gly | Asp | Ser |     |
|    | 65                         |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     | 80  |     |     |
| 5  | Gln                        | Leu | Ser | Thr | Trp | Leu | Tyr | Gly | Ile | Ala | Leu | Asn | Leu | Val | Arg | Asn |     |
|    |                            |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
|    | His                        | Leu | Ser | Arg | Ala | Pro | Glu | Arg | Arg | Tyr | Glu | Phe | Thr | Ser | Asp | Ala |     |
|    |                            |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
| 10 | Ser                        | Leu | Gly | Val | Met | Pro | Cys | Ser | Ala | Pro | Asn | Pro | Glu | Ala | Val | Thr |     |
|    |                            |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
|    | Glu                        | Gln | Arg | Gln | Arg | Met | Arg | Leu | Leu | Arg | Glu | Ala | Leu | Glu | Gln | Leu |     |
|    |                            |     | 130 |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |
|    | Pro                        | Glu | Ser | Met | Arg | Asp | Val | Ile | Leu | Met | Val | Gly | Val | Glu | Glu | Leu |     |
|    | 145                        |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |     |
| 15 | Ser                        | Tyr | Glu | Glu | Ala | Ala | Ala | Leu | Leu | Ser | Val | Pro | Val | Gly | Thr | Ile |     |
|    |                            |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
|    | Arg                        | Ser | Arg | Leu | Ser | Arg | Ala | Arg | Cys | Ala | Leu | Arg | Glu | Ala | Leu | Arg |     |
|    |                            |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |
| 20 | Glu                        | Arg | Gly | Tyr | Asp | Ser | Val | Pro |     |     |     |     |     |     |     |     |     |
|    |                            |     | 195 |     |     |     | 200 |     |     |     |     |     |     |     |     |     |     |
|    | <210> 41                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 1098                 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> DNA                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 25 | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <220>                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <221> CDS                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <222> (1)...(1098)         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 30 | <400> 41                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | atg                        | act | cgt | atc | gat | gcc | gcc | ccc | aat | ccc | ttc | cac | gcc | gcc | atg | cag | 48  |
|    | Met                        | Thr | Arg | Ile | Asp | Ala | Ala | Pro | Asn | Pro | Phe | His | Ala | Ala | Met | Gln |     |
|    | 1                          |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |     |
| 35 | ggg                        | cgc | cac | gac | gcc | tcg | gcc | aac | acc | tcc | tcc | ggc | tgg | ctg | caa | ggc | 96  |
|    | Gly                        | Arg | His | Asp | Ala | Ser | Ala | Asn | Thr | Ser | Ser | Gly | Trp | Leu | Gln | Gly |     |
|    |                            |     |     | 20  |     |     |     | 25  |     |     |     |     |     | 30  |     |     |     |
| 40 | cag                        | cgc | atc | gca | ccg | gcg | ccc | acc | ggc | ata | tcg | ctg | gcg | gac | gcg | gcc | 144 |
|    | Gln                        | Arg | Ile | Ala | Pro | Ala | Pro | Thr | Gly | Ile | Ser | Leu | Ala | Asp | Ala | Ala |     |
|    |                            |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |     |
| 45 | gag                        | gag | ctc | agc | ctg | cac | atg | gcg | cag | gct | gcc | gag | gaa | aag | cat | cac | 192 |
|    | Glu                        | Glu | Leu | Ser | Leu | His | Met | ala | Gln | Ala | Ala | Glu | Glu | Lys | His | His |     |
|    |                            | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |     |
| 50 | tcc                        | gaa | cgc | aag | gtc | acg | gcc | gaa | cgt | ccg | atg | ctc | tgg | ctg | gac | gcg | 240 |
|    | Ser                        | Glu | Arg | Lys | Val | Thr | Ala | Glu | Arg | Pro | Met | Leu | Trp | Leu | Asp | Ala |     |
|    | 65                         |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |     |
| 55 | gcg                        | cag | ctt | gcg | gaa | ctg | ttt | tcc | cac | acc | cac | gac | ccc | gac | gcg | cag | 288 |
|    | Ala                        | Gln | Leu | Ala | Glu | Leu | Phe | Ser | His | Thr | His | Asp | Pro | Asp | Ala | Gln |     |
|    |                            |     |     | 85  |     |     |     |     |     | 90  |     |     |     | 95  |     |     |     |
| 60 | gca                        | aaa | ctg | gaa | gcc | ctg | acc | gcc | gag | ctg | ctg | cgc | ggc | cgg | ggc | gcc | 336 |
|    | Ala                        | Lys | Leu | Glu | Ala | Leu | Thr | Ala | Glu | Leu | Leu | Arg | Gly | Arg | Gly | Ala |     |
|    |                            |     |     | 100 |     |     |     | 105 |     |     |     |     | 110 |     |     |     |     |
| 60 | ccc                        | atg | cag | ctg | gcc | gcg | caa | gcg | ttt | ccc | ggt | gtc | acg | cag | caa | tac | 384 |

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|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
|    | Pro | Met | Gln | Leu | Ala | Ala | Gln | Ala | Phe | Pro | Gly | Val | Thr | Gln | Gln | Tyr |      |
|    |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |      |
| 5  | ctc | gcg | ctg | cag | cac | gcg | ctg | cag | cgc | ggc | gag | cac | gag | gac | gcc | gcg | 432  |
|    | Leu | Ala | Leu | Gln | His | Ala | Leu | Gln | Arg | Gly | Glu | His | Glu | Asp | Ala | Ala |      |
|    |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |      |
| 10 | cgc | cac | gcg | ctc | gaa | gcc | ctg | cgc | gat | gca | ttg | gcc | gac | ctg | gag | ctc | 480  |
|    | Pro | His | Ala | Leu | Glu | Ala | Leu | Arg | Asp | Ala | Leu | Ala | Asp | Leu | Glu | Leu |      |
|    | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |      |
| 15 | gcc | cat | ggc | ccc | gaa | atc | cgc | gcc | ggc | atc | aac | acc | ctg | ccc | acg | gcc | 528  |
|    | Ala | His | Gly | Pro | Glu | Ile | Arg | Ala | Gly | Ile | Asn | Thr | Leu | Pro | Thr | Ala |      |
|    |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |      |
| 20 | ggc | gca | ttc | gcg | cgt | tcc | gct | gac | gag | ctg | gcc | ggc | ttc | cag | cac | gcg | 576  |
|    | Gly | Ala | Phe | Ala | Arg | Ser | Ala | Asp | Glu | Leu | Ala | Gly | Phe | Gln | His | Ala |      |
|    |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |      |
| 25 | tac | cgc | gac | atc | gcc | ctg | ggc | cag | ctg | tgc | ttg | gcg | cgc | acg | ctg | gac | 624  |
|    | Tyr | Arg | Asp | Ile | Ala | Leu | Gly | Gln | Leu | Ser | Leu | Ala | Arg | Thr | Leu | Asp |      |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |      |
| 30 | ctg | gtg | ctg | gaa | cgc | tat | ggg | aac | gac | gac | atc | cac | ggc | gcg | ctg | ggc | 672  |
|    | Leu | Val | Leu | Glu | Arg | Tyr | Gly | Asn | Asp | Asp | Ile | His | Gly | Ala | Leu | Gly |      |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |      |
| 35 | gcg | ctg | att | cag | gcg | ctg | gga | cac | gac | ctg | gcc | gcg | gcg | aca | ccg | tgc | 720  |
|    | Ala | Leu | Ile | Gln | Ala | Leu | Gly | His | Asp | Leu | Ala | Ala | Ala | Thr | Pro | Ser |      |
|    | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |      |
| 40 | acg | gac | ggc | gtc | agg | ctg | caa | gtg | ttg | gcg | agc | gat | ctc | tat | caa | gtc | 768  |
|    | Thr | Asp | Gly | Val | Arg | Leu | Gln | Val | Leu | Ala | Ser | Asp | Leu | Tyr | Gln | Val |      |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |      |
| 45 | gag | gtg | gcc | gcc | acg | gta | ctg | gag | gaa | tgc | aac | gcc | ctg | aaa | caa | cgg | 816  |
|    | Glu | Val | Ala | Ala | Thr | Val | Leu | Glu | Glu | Cys | Asn | Ala | Leu | Lys | Gln | Arg |      |
|    |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |      |
| 50 | ttg | ggc | aac | gca | ggc | tgc | cag | gag | tgt | gcg | gac | gcc | cag | ggc | ctg | atg | 864  |
|    | Leu | Gly | Asn | Ala | Gly | Ser | Gln | Glu | Cys | Ala | Asp | Ala | Gln | Gly | Leu | Met |      |
|    |     |     | 275 |     |     |     | 280 |     |     |     |     |     | 285 |     |     |     |      |
| 55 | cgc | gat | ctt | gtg | gga | atc | agc | gag | gac | aaa | tgg | att | gcg | ccc | gcg | cgc | 912  |
|    | Arg | Asp | Leu | Val | Gly | Ile | Ser | Glu | Asp | Lys | Trp | Ile | Ala | Pro | Ala | Arg |      |
|    |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |      |
| 60 | ttc | gag | aag | ctg | gcc | gag | cgc | cac | ggt | gcg | aac | gcc | ctc | tcc | gag | cgc | 960  |
|    | Phe | Glu | Lys | Leu | Ala | Glu | Arg | His | Gly | Ala | Asn | Ala | Leu | Ser | Glu | Arg |      |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |      |
| 65 | atc | gca | ttc | ctc | ggt | ggc | gta | cgc | cag | att | ctc | aaa | gac | ctg | ccc | acg | 1008 |
|    | Ile | Ala | Phe | Leu | Gly | Gly | Val | Arg | Gln | Ile | Leu | Lys | Asp | Leu | Pro | Thr |      |
|    |     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |      |
| 70 | cag | atc | tac | gcc | gac | atg | gac | gtg | cgc | gcc | acc | gtc | ctg | gcg | gcc | gcg | 1056 |
|    | Gln | Ile | Tyr | Ala | Asp | Met | Asp | Val | Arg | Ala | Thr | Val | Leu | Ala | Ala | Ala |      |
|    |     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |      |
| 75 | cag | gac | gcg | ctg | gac | aac | gcg | ata | gca | atg | gag | aac | gca | tga |     |     | 1098 |



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Gln Asp Ala Leu Asp Asn Ala Ile Ala Met Glu Asn Ala \*

355 360 365

5 <210> 42  
 <211> 365  
 <212> PRT  
 <213> Bordetella pertussis

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 Gly Arg His Asp Ala Ser Ala Asn Thr Ser Ser Gly Trp Leu Gln Gly  
 20 25 30  
 15 Gln Arg Ile Ala Pro Ala Pro Thr Gly Ile Ser Leu Ala Asp Ala Ala  
 35 40 45  
 Glu Glu Leu Ser Leu His Met ala Gln Ala Ala Glu Lys His His  
 50 55 60  
 Ser Glu Arg Lys Val Thr Ala Glu Arg Pro Met Leu Trp Leu Asp Ala  
 65 70 75 80  
 20 Ala Gln Leu Ala Glu Leu Phe Ser His Thr His Asp Pro Asp Ala Gln  
 85 90 95  
 Ala Lys Leu Glu Ala Leu Thr Ala Glu Leu Arg Gly Arg Gly Ala  
 100 105 110  
 25 Pro Met Gln Leu Ala Ala Gln Ala Phe Pro Gly Val Thr Gln Gln Tyr  
 115 120 125  
 Leu Ala Leu Gln His Ala Leu Gln Arg Gly Glu His Glu Asp Ala Ala  
 130 135 140  
 Pro His Ala Leu Glu Ala Leu Arg Asp Ala Leu Ala Asp Leu Glu Leu  
 145 150 155 160  
 30 Ala His Gly Pro Glu Ile Arg Ala Gly Ile Asn Thr Leu Pro Thr Ala  
 165 170 175  
 Gly Ala Phe Ala Arg Ser Ala Asp Glu Leu Ala Gly Phe Gln His Ala  
 180 185 190  
 35 Tyr Arg Asp Ile Ala Leu Gly Gln Leu Ser Leu Ala Arg Thr Leu Asp  
 195 200 205  
 Leu Val Leu Glu Arg Tyr Gly Asn Asp Asp Ile His Gly Ala Leu Gly  
 210 215 220  
 Ala Leu Ile Gln Ala Leu Gly His Asp Leu Ala Ala Thr Pro Ser  
 225 230 235 240  
 40 Thr Asp Gly Val Arg Leu Gln Val Leu Ala Ser Asp Leu Tyr Gln Val  
 245 250 255  
 Glu Val Ala Ala Thr Val Leu Glu Glu Cys Asn Ala Leu Lys Gln Arg  
 260 265 270  
 45 Leu Gly Asn Ala Gly Ser Gln Glu Cys Ala Asp Ala Gln Gly Leu Met  
 275 280 285  
 Arg Asp Leu Val Gly Ile Ser Glu Asp Lys Trp Ile Ala Pro Ala Arg  
 290 295 300  
 Phe Glu Lys Leu Ala Glu Arg His Gly Ala Asn Ala Leu Ser Glu Arg  
 305 310 315 320  
 50 Ile Ala Phe Leu Gly Gly Val Arg Gln Ile Leu Lys Asp Leu Pro Thr  
 325 330 335  
 Gln Ile Tyr Ala Asp Met Asp Val Arg Ala Thr Val Leu Ala Ala Ala  
 340 345 350  
 55 Gln Asp Ala Leu Asp Asn Ala Ile Ala Met Glu Asn Ala  
 355 360 365

60 <210> 43  
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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <220>                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <221> CDS                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5  | <222> (1)...(588)          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <400> 43                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 10 | atg                        | ccc | ttg | gcg | ggg | tcc | tgc | ggc | cgg | acg | tcc | gaa | aac | cgg | gat | att | 48  |
|    | Met                        | Pro | Leu | Ala | Gly | Ser | Cys | Gly | Arg | Thr | Ser | Glu | Asn | Arg | Asp | Ile |     |
|    | 1                          |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|    | ccg                        | aga | agc | aat | cgg | ctg | gcg | cct | tcc | aga | ctg | tgc | gca | tac | cat | tgc | 96  |
|    | Pro                        | Arg | Ser | Asn | Arg | Leu | Ala | Pro | Ser | Arg | Leu | Cys | Ala | Tyr | His | Cys |     |
|    |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| 15 | cct                        | ctt | ttg | cca | cgc | att | tcg | agc | gta | tgg | ttt | ccc | ttg | cgc | ccg | acg | 144 |
|    | Pro                        | Leu | Leu | Pro | Arg | Ile | Ser | Ser | Val | Trp | Phe | Pro | Leu | Arg | Pro | Thr |     |
|    |                            |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
| 20 | cag                        | gct | cgc | ctc | gcc | atg | acc | gac | acg | gca | tac | cac | caa | ctc | atc | gcc | 192 |
|    | Gln                        | Ala | Arg | Leu | Ala | Met | Thr | Asp | Thr | Ala | Tyr | His | Gln | Leu | Ile | Ala |     |
|    |                            | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |
| 25 | gat                        | ttc | ggc | cgc | ctc | atc | ggc | atc | gac | tcg | ctc | aac | ccc | ggt | gcc | ggc | 240 |
|    | Asp                        | Phe | Gly | Arg | Leu | Ile | Gly | Ile | Asp | Ser | Leu | Asn | Pro | Gly | Ala | Gly |     |
|    | 65                         |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
|    | ggc                        | ctg | tgt | cag | ttg | att | ttc | gaa | ccg | tgc | gca | ccg | gtc | ttc | atc | gca | 288 |
|    | Gly                        | Leu | Cys | Gln | Leu | Ile | Phe | Glu | Pro | Cys | Ala | Pro | Val | Phe | Ile | Ala |     |
|    |                            |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
| 30 | ccg                        | gtg | cac | gcc | cgg | acg | gaa | atc | atg | att | tcc | tgc | gtg | ctg | ggc | acg | 336 |
|    | Pro                        | Val | His | Ala | Arg | Thr | Glu | Ile | Met | Ile | Ser | Cys | Val | Leu | Gly | Thr |     |
|    |                            |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
| 35 | gcg                        | gac | gcg | gcc | aac | ccg | gca | agc | atg | gcc | cga | gcc | aac | ttc | atg | cag | 384 |
|    | Ala                        | Asp | Ala | Ala | Asn | Pro | Ala | Ser | Met | ala | Arg | Ala | Asn | Phe | Met | Gln |     |
|    |                            |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
| 40 | gcc                        | ggc | agc | ggc | gtc | gtg | gcc | tgc | atc | ggc | ggc | gat | ggg | ttg | ttc | tat | 432 |
|    | Ala                        | Gly | Ser | Gly | Val | Val | Ala | Cys | Ile | Gly | Gly | Asp | Gly | Leu | Phe | Tyr |     |
|    |                            | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |
| 45 | ctg                        | cag | cag | gcc | ata | ccc | ctg | tcg | cgc | gcc | acg | ccc | gca | atc | ctg | ctc | 480 |
|    | Leu                        | Gln | Gln | Ala | Ile | Pro | Leu | Ser | Arg | Ala | Thr | Pro | Ala | Ile | Leu | Leu |     |
|    | 145                        |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
|    | gat                        | cac | tgt | gag | cgt | ctg | ctg | cag | gaa | gcc | tcg | cgc | tgg | cgc | gtc | ggc | 528 |
|    | Asp                        | His | Cys | Glu | Arg | Leu | Leu | Gln | Glu | Ala | Ser | Arg | Trp | Arg | Val | Gly |     |
|    |                            |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
| 50 | gac                        | cac | gac | ggc | tgc | gcc | acc | tcg | gcc | ccg | aat | atc | gcc | gcg | ctg | acg | 576 |
|    | Asp                        | His | Asp | Gly | Cys | Ala | Thr | Ser | Ala | Pro | Asn | Ile | Ala | Ala | Leu | Thr |     |
|    |                            |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |
| 55 | cgc                        | ggc | gtc | tag |     |     |     |     |     |     |     |     |     |     |     |     | 588 |
|    | Arg                        | Gly | Val | *   |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    |                            |     | 195 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 60 |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

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 <213> Bordetella pertussis  
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 Pro Arg Ser Asn Arg Leu Ala Pro Ser Arg Leu Cys Ala Tyr His Cys  
 10 20 25 30  
 Pro Leu Leu Pro Arg Ile Ser Ser Val Trp Phe Pro Leu Arg Pro Thr  
 35 40 45  
 Gln Ala Arg Leu Ala Met Thr Asp Thr Ala Tyr His Gln Leu Ile Ala  
 50 55 60  
 15 Asp Phe Gly Arg Leu Ile Gly Ile Asp Ser Leu Asn Pro Gly Ala Gly  
 65 70 75 80  
 Gly Leu Cys Gln Leu Ile Phe Glu Pro Cys Ala Pro Val Phe Ile Ala  
 85 90 95  
 20 Pro Val His Ala Arg Thr Glu Ile Met Ile Ser Cys Val Leu Gly Thr  
 100 105 110  
 Ala Asp Ala Ala Asn Pro Ala Ser Met ala Arg Ala Asn Phe Met Gln  
 115 120 125  
 Ala Gly Ser Gly Val Val Ala Cys Ile Gly Gly Asp Gly Leu Phe Tyr  
 130 135 140  
 25 Leu Gln Gln Ala Ile Pro Leu Ser Arg Ala Thr Pro Ala Ile Leu Leu  
 145 150 155 160  
 Asp His Cys Glu Arg Leu Leu Gln Glu Ala Ser Arg Trp Arg Val Gly  
 165 170 175  
 30 Asp His Asp Gly Cys Ala Thr Ser Ala Pro Asn Ile Ala Ala Leu Thr  
 180 185 190  
 Arg Gly Val  
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 45 Met Met Pro His Thr Leu Pro Ser Pro Ser Leu Gln Val Arg Glu Leu  
 1 5 10 15  
 ctg caa ttg ctt gcg cac cac tac cag ttg cag cgc caa tgg agc aag 96  
 50 Leu Gln Leu Leu Ala His His Tyr Gln Leu Gln Arg Gln Trp Ser Lys  
 20 25 30  
 acg gtt gcc ctg ctg gcg gcc ctg gat gcc ctg gac gcg atc gac agc 144  
 Thr Val Ala Leu Leu Ala Ala Leu Asp Ala Leu Asp Ala Ile Asp Ser  
 35 40 45  
 55 cag tcc ctg ctg gcc ctg gcg ctg ggc tat ctg cac cag ggc gaa ccg 192  
 Gln Ser Leu Leu Ala Leu Ala Leu Gly Tyr Leu His Gln Gly Glu Pro  
 50 55 60  
 60 cgc atg gcc ttg gtc acg ctg gac aag cgc gca ctg cgc gcc aca ccc 240

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Arg Met ala Leu Val Thr Leu Asp Lys Arg Ala Leu Arg Ala Thr Pro
65              70              75              80

5  gat gcc gcc ggc cat ctg gtc cgg gcg cag gcc atg cag gcg ctg aac      288
   Asp Ala Ala Gly His Leu Val Arg Ala Gln Ala Met Gln Ala Leu Asn
                        85              90              95

10 cga ccc gac gac gcc cgc cag gcc atg cgt gac tat atg gcg cta cgg      336
   Arg Pro Asp Asp Ala Arg Gln Ala Met Arg Asp Tyr Met ala Leu Arg
                        100              105              110

15 gcg gcg tct tgc gcg agc gcg acc ccg cca tga      369
   Ala Ala Ser Cys Ala Ser Ala Thr Pro Pro *
                        115              120

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   <212> PRT
   <213> Bordetella pertussis

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1  5 10 15
25 Leu Gln Leu Leu Ala His His Tyr Gln Leu Gln Arg Gln Trp Ser Lys
   20 25 30
   Thr Val Ala Leu Leu Ala Ala Leu Asp Ala Leu Asp Ala Ile Asp Ser
   35 40 45
30 Gln Ser Leu Leu Ala Leu Ala Leu Gly Tyr Leu His Gln Gly Glu Pro
   50 55 60
   Arg Met ala Leu Val Thr Leu Asp Lys Arg Ala Leu Arg Ala Thr Pro
   65 70 75 80
   Asp Ala Ala Gly His Leu Val Arg Ala Gln Ala Met Gln Ala Leu Asn
   85 90 95
35 Arg Pro Asp Asp Ala Arg Gln Ala Met Arg Asp Tyr Met ala Leu Arg
   100 105 110
   Ala Ala Ser Cys Ala Ser Ala Thr Pro Pro
   115 120

40 <210> 47
   <211> 411
   <212> DNA
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45 <220>
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50 atg tcc agc gcc gta ccc ggc atg cat gcc atg cac ctc ggc ctg gag      48
   Met Ser Ser Ala Val Pro Gly Met His Ala Met His Leu Gly Leu Glu
   1  5 10 15

55 cgc ggc gtc gac cac atc gtg cgc ggt ccc cgc tgc gag ccc gcc ccc      96
   Arg Gly Val Asp His Ile Val Arg Gly Pro Arg Cys Glu Pro Ala Pro
   20 25 30

60 acc ctg cca ccc gag cgc tgg ctc gaa ccg ccc gcc acc ggc gcg gtc      144
   Thr Leu Pro Pro Glu Arg Trp Leu Glu Pro Pro Ala Thr Thr Gly Ala Val
   35 40 45

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[illegible]

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    <220>
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    Met Gly Ser Pro Arg Arg Arg Asn His Leu Pro Thr Gly Ala Val Ser
    1          5          10          15

    gtc gcg cgc gcg gtc atg gtt ccc gga aac ggg cgc gat att ggg caa      96
    Val Ala Arg Ala Val Met Val Pro Gly Asn Gly Arg Asp Ile Gly Gln
          20          25          30

    ttc gca gcc tgg aac ttg ccg cgg gcg cag ggt tac tca gca tgc gtc      144
    Phe Ala Ala Trp Asn Leu Pro Arg Ala Gln Gly Tyr Ser Ala Cys Val
          35          40          45

    ttt caa ctc gaa gga gct ctc atg agc att gat ctc gga gtt tca ctc      192
    Phe Gln Leu Glu Gly Ala Leu Met Ser Ile Asp Leu Gly Val Ser Leu
    50          55          60

    acg tcg cag gcc ggc ggc ctg caa ggc atc gac ctc aag agc atg gat      240
    Thr Ser Gln Ala Gly Gly Leu Gln Gly Ile Asp Leu Lys Ser Met Asp
    65          70          75          80

    atc cag act ctc atg gtg tat gtg cag ggt cgt cgc gcc gaa ctc ctc      288
    Ile Gln Thr Leu Met Val Tyr Val Gln Gly Arg Arg Ala Glu Leu Leu
          85          90          95

    acg gct caa atg cag acc cag gcc gaa gtg gtg cag aag gcc aat gaa      336
    Thr Ala Gln Met Gln Thr Gln Ala Glu Val Val Gln Lys Ala Asn Glu
    100          105          110

    cgc atg gcg cag ctc aac gag gtc ctg tcc gcg ctg tcc cgg gcc aag      384
    Arg Met Ala Gln Leu Asn Glu Val Leu Ser Ala Leu Ser Arg Ala Lys
    115          120          125

    gcc gag ttt ccg ccc aat ccg aag ccg ggc gac acc atc ccg ggc tgg      432
    Ala Glu Phe Pro Pro Asn Pro Lys Pro Gly Asp Thr Ile Pro Gly Trp
    130          135          140

    gac agc cag aag atc agc cgg atc gag gtt cct ctc aat gat gcg ctg      480
    Asp Ser Gln Lys Ile Ser Arg Ile Glu Val Pro Leu Asn Asp Ala Leu
    145          150          155          160

    cgt gcc gcc ggc ctg acg ggc atg ttc gaa gcg cgc gat ggc cgg gtg      528
    Arg Ala Ala Gly Leu Thr Gly Met Phe Glu Ala Arg Asp Gly Arg Val
    165          170          175

    acc ggc ccc gac ggc cgg ggt acg cag gtc gtg aac ggc acg ggc gtc      576
    Thr Gly Pro Asp Gly Arg Gly Thr Gln Val Val Asn Gly Thr Gly Val
    180          185          190

    atg gcc ggt tcc acg acc tat aag gaa ctc gaa agt gcc tac acc acc      624
    Met Ala Gly Ser Thr Thr Tyr Lys Glu Leu Glu Ser Ala Tyr Thr Thr
    195          200          205

    gta aag ggg atg ctg gat acg gcg tcc aat acg caa cag atg gac atg      672

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|    |                            |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Val                        | Lys | Gly | Met | Leu | Asp | Thr | Ala | Ser | Asn | Thr | Gln | Gln | Met | Asp | Met |     |
|    | 210                        |     |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
| 5  | atc                        | agg | ctg | cag | gcc | gcc | agc | aac | aag | cgc | aac | gag | gct | ttc | gag | gtc | 720 |
|    | Ile                        | Arg | Leu | Gln | Ala | Ala | Ser | Asn | Lys | Arg | Asn | Glu | Ala | Phe | Glu | Val |     |
|    | 225                        |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |
| 10 | atg                        | acc | aac | acc | gag | aag | cgg | cgc | agc | gac | ttg | aac | agc | tcc | atc | acc | 768 |
|    | Met                        | Thr | Asn | Thr | Glu | Lys | Arg | Arg | Ser | Asp | Leu | Asn | Ser | Ser | Ile | Thr |     |
|    |                            |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |     |
| 15 | agc                        | aac | atg | cgc | taa |     |     |     |     |     |     |     |     |     |     |     | 783 |
|    | Ser                        | Asn | Met | Arg | *   |     |     |     |     |     |     |     |     |     |     |     |     |
|    |                            |     |     |     | 260 |     |     |     |     |     |     |     |     |     |     |     |     |
| 20 | <210> 52                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <211> 260                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <212> PRT                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | <213> Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 25 | <400> 52                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | Met                        | Gly | Ser | Pro | Arg | Arg | Arg | Asn | His | Leu | Pro | Thr | Gly | Ala | Val | Ser |     |
|    | 1                          |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|    | Val                        | Ala | Arg | Ala | Val | Met | Val | Pro | Gly | Asn | Gly | Arg | Asp | Ile | Gly | Gln |     |
|    |                            |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
|    | Phe                        | Ala | Ala | Trp | Asn | Leu | Pro | Arg | Ala | Gln | Gly | Tyr | Ser | Ala | Cys | Val |     |
|    |                            |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |     |
| 30 | Phe                        | Gln | Leu | Glu | Gly | Ala | Leu | Met | Ser | Ile | Asp | Leu | Gly | Val | Ser | Leu |     |
|    | 50                         |     |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |
|    | Thr                        | Ser | Gln | Ala | Gly | Gly | Leu | Gln | Gly | Ile | Asp | Leu | Lys | Ser | Met | Asp |     |
|    | 65                         |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |     |
|    | Ile                        | Gln | Thr | Leu | Met | Val | Tyr | Val | Gln | Gly | Arg | Arg | Ala | Glu | Leu | Leu |     |
|    |                            |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |     |
| 35 | Thr                        | Ala | Gln | Met | Gln | Thr | Gln | Ala | Glu | Val | Val | Gln | Lys | Ala | Asn | Glu |     |
|    |                            |     |     | 100 |     |     |     | 105 |     |     |     |     |     | 110 |     |     |     |
|    | Arg                        | Met | Ala | Gln | Leu | Asn | Glu | Val | Leu | Ser | Ala | Leu | Ser | Arg | Ala | Lys |     |
|    |                            |     | 115 |     |     |     | 120 |     |     |     |     |     | 125 |     |     |     |     |
| 40 | Ala                        | Glu | Phe | Pro | Pro | Asn | Pro | Lys | Pro | Gly | Asp | Thr | Ile | Pro | Gly | Trp |     |
|    | 130                        |     |     |     |     | 135 |     |     |     |     |     | 140 |     |     |     |     |     |
|    | Asp                        | Ser | Gln | Lys | Ile | Ser | Arg | Ile | Glu | Val | Pro | Leu | Asn | Asp | Ala | Leu |     |
|    | 145                        |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |     |
|    | Arg                        | Ala | Ala | Gly | Leu | Thr | Gly | Met | Phe | Glu | Ala | Arg | Asp | Gly | Arg | Val |     |
|    |                            |     |     | 165 |     |     |     |     | 170 |     |     |     |     |     | 175 |     |     |
| 45 | Thr                        | Gly | Pro | Asp | Gly | Arg | Gly | Thr | Gln | Val | Val | Asn | Gly | Thr | Gly | Val |     |
|    |                            |     |     | 180 |     |     |     | 185 |     |     |     |     |     | 190 |     |     |     |
|    | Met                        | Ala | Gly | Ser | Thr | Thr | Tyr | Lys | Glu | Leu | Glu | Ser | Ala | Tyr | Thr | Thr |     |
|    |                            |     | 195 |     |     |     | 200 |     |     |     |     |     | 205 |     |     |     |     |
| 50 | Val                        | Lys | Gly | Met | Leu | Asp | Thr | Ala | Ser | Asn | Thr | Gln | Gln | Met | Asp | Met |     |
|    | 210                        |     |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
|    | Ile                        | Arg | Leu | Gln | Ala | Ala | Ser | Asn | Lys | Arg | Asn | Glu | Ala | Phe | Glu | Val |     |
|    | 225                        |     |     |     | 230 |     |     |     |     |     | 235 |     |     |     | 240 |     |     |
|    | Met                        | Thr | Asn | Thr | Glu | Lys | Arg | Arg | Ser | Asp | Leu | Asn | Ser | Ser | Ile | Thr |     |
|    |                            |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |     |
| 55 | Ser                        | Asn | Met | Arg |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    |                            |     |     | 260 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 60 | <210> 53                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
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    <213> Bordetella pertussis
    <220>
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    Met Gln Glu Gln Gly Ile Gln Ser Ile Met Arg Ala Ala Glu Glu Leu
10    1          5          10          15

    gtc gag cag acc cgc cag gcg ttg tac agc gtc gac gag atc tac gcc      96
    Val Glu Gln Thr Arg Gln Ala Leu Tyr Ser Val Asp Glu Ile Tyr Ala
    20          25          30

15    cac gtt ggc gtc gac ccc gct cgc ctg cgc aat ctg gcg gtc gag cag      144
    His Val Gly Val Asp Pro Ala Arg Leu Arg Asn Leu Ala Val Glu Gln
    35          40          45

20    gcc agg ata gag gcc gag gcc cag gcg gcg ttc cgt gat gac ctc gcg      192
    Ala Arg Ile Glu Ala Glu Ala Gln Ala Ala Phe Arg Asp Asp Leu Ala
    50          55          60

25    gac atc gag cgc gag gcg gcg cgc gtc aag gcg gcc tgc acc gat gcg      240
    Asp Ile Glu Arg Glu Ala Ala Arg Val Lys Ala Ala Cys Thr Asp Ala
    65          70          75          80

    ccg cag gcc cgc agg gtg ctt cac aac cac gtc tga      276
30    Pro Gln Ala Arg Arg Val Leu His Asn His Val *
    85          90

    <210> 54
    <211> 91
35    <212> PRT
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    <400> 54
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    Val Glu Gln Thr Arg Gln Ala Leu Tyr Ser Val Asp Glu Ile Tyr Ala
    20          25          30
    His Val Gly Val Asp Pro Ala Arg Leu Arg Asn Leu Ala Val Glu Gln
    35          40          45
45    Ala Arg Ile Glu Ala Glu Ala Gln Ala Ala Phe Arg Asp Asp Leu Ala
    50          55          60
    Asp Ile Glu Arg Glu Ala Ala Arg Val Lys Ala Ala Cys Thr Asp Ala
    65          70          75          80
50    Pro Gln Ala Arg Arg Val Leu His Asn His Val
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|    | <400> 55 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5  | atg      | tct | ggt | tct | ccg | act | tcg | ccc | ggc | tct | ttc | ggg | gcc | ggc | cct | gtc | 48  |
|    | Met      | Ser | Val | Ser | Pro | Thr | Ser | Pro | Gly | Ser | Phe | Gly | Ala | Gly | Pro | Val |     |
|    | 1        |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
|    | ttt      | gac | tcc | gaa | ttg | cag | gcc | ccg | ggc | ccg | tcg | gcg | cag | cgt | cgc | ggc | 96  |
|    | Phe      | Asp | Ser | Glu | Leu | Gln | Ala | Pro | Ala | Pro | Ser | Ala | Gln | Arg | Arg | Gly |     |
|    |          |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| 10 | ggt      | gcg | gcg | cct | gtg | ccg | ccg | ccc | gtc | gat | ccg | cgc | ggc | gtc | gag | ccg | 144 |
|    | Gly      | Ala | Ala | Pro | Val | Pro | Pro | Pro | Val | Asp | Arg | Arg | Gly | Val | Glu | Pro |     |
|    |          |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
| 15 | gga      | gat | ccc | acg | ctg | ggc | atg | ctg | ccc | gcg | cca | gat | ttg | ctc | gcg | ggg | 192 |
|    | Gly      | Asp | Pro | Thr | Leu | Gly | Met | Leu | Pro | Ala | Pro | Asp | Leu | Leu | Ala | Gly |     |
|    |          | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |
| 20 | ggc      | gcc | gtc | agc | cgc | acc | cgc | gcg | gcg | ctc | gac | gat | ctg | gac | gcc | gca | 240 |
|    | Gly      | Ala | Val | Ser | Arg | Thr | Arg | Ala | Ala | Leu | Asp | Asp | Leu | Asp | Ala | Ala |     |
|    | 65       |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
| 25 | cgg      | ctc | ggt | gaa | gac | atc | tac | gcc | ttg | atg | gcg | gtg | ttg | caa | cag | gcc | 288 |
|    | Arg      | Leu | Gly | Glu | Asp | Ile | Tyr | Ala | Leu | Met | ala | Val | Leu | Gln | Gln | Ala |     |
|    |          |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
|    | agt      | cag | cag | atg | cgg | gac | gcc | gcc | cgt | atc | gct | cgt | gat | gcc | gag | gct | 336 |
|    | Ser      | Gln | Gln | Met | Arg | Asp | Ala | Ala | Arg | Ile | Ala | Arg | Asp | Ala | Glu | Ala |     |
|    |          |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
| 30 | acg      | cgg | caa | acg | cag | gct | ctc | ggc | gat | gcg | gcc | agc | cag | atg | cgc | cag | 384 |
|    | Thr      | Arg | Gln | Thr | Gln | Ala | Leu | Gly | Asp | Ala | Ala | Ser | Gln | Met | Arg | Gln |     |
|    |          |     |     | 115 |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
| 35 | gcg      | gcg | agc | gag | cgc | atg | gcc | gga | gcg | atc | gtg | gcg | ggc | gcc | atg | cag | 432 |
|    | Ala      | Ala | Ser | Glu | Arg | Met | ala | Gly | Ala | Ile | Val | Ala | Gly | Ala | Met | Gln |     |
|    |          | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |
| 40 | ata      | gcg | ggt | ggt | ttc | gtg | cag | ctg | ggg | gcg | ggc | ctg | gca | gcg | ggt | ttg | 480 |
|    | Ile      | Ala | Gly | Gly | Phe | Val | Gln | Leu | Gly | Ala | Gly | Leu | Ala | Ala | Gly | Leu |     |
|    | 145      |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
| 45 | cag      | gcc | atg | ggt | ggc | gct | gct | gcg | caa | gcc | aag | ggc | gcc | gca | ttc | tcc | 528 |
|    | Gln      | Ala | Met | Gly | Gly | Ala | Ala | Ala | Gln | Ala | Lys | Gly | Ala | Ala | Phe | Ser |     |
|    |          |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
|    | gag      | cag | gcc | tcg | aca | agc | cgc | aag | gtg | gcg | gcc | ggc | ttg | cac | gat | gcc | 576 |
|    | Glu      | Gln | Ala | Ser | Thr | Ser | Arg | Lys | Val | Ala | Ala | Gly | Leu | His | Asp | Ala |     |
|    |          |     |     | 180 |     |     |     | 185 |     |     |     |     |     | 190 |     |     |     |
| 50 | ccc      | gag | ctg | cag | gca | acg | gtg | cag | gcc | cgc | gca | acc | cag | ctc | gaa | gcg | 624 |
|    | Pro      | Glu | Leu | Gln | Ala | Thr | Val | Gln | Ala | Arg | Ala | Thr | Gln | Leu | Glu | Ala |     |
|    |          |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
| 55 | caa      | gcg | gcc | tcg | ttt | ggt | gcg | gac | gcg | gct | cgt | tcg | tcg | gca | aag | tcg | 672 |
|    | Gln      | Ala | Ala | Ser | Phe | Gly | Ala | Asp | Ala | Ala | Arg | Ser | Ser | Ala | Lys | Ser |     |
|    |          | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
| 60 | cag      | cgc | gta | tcg | agc | gtt | gcc | cag | gcc | ggc | gcc | gca | gcg | gcc | ggc | ggt | 720 |
|    | Gln      | Arg | Val | Ser | Ser | Val | Ala | Gln | Ala | Gly | Ala | Ala | Ala | Ala | Gly | Gly |     |
|    | 225      |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |

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|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
|    | Ala | Ser | Val | Val | Thr | Gly | Ala | Ala | Ala | Thr | Pro | Met | Leu | Val | Leu | Ser |      |
|    |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |      |
| 5  | ggc | atg | gca | ttg | gtc | agc | gcc | gtg | aca | tcg | ctg | gcc | gac | cag | ata | tcg | 576  |
|    | Gly | Met | ala | Leu | Val | Ser | Ala | Val | Thr | Ser | Leu | Ala | Asp | Gln | Ile | Ser |      |
|    |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |      |
| 10 | cga | gag | gcg | gga | ggg | ccg | cct | atc | agc | ctg | ggc | ggg | ttt | ctc | tcc | ggg | 624  |
|    | Arg | Glu | Ala | Gly | Gly | Pro | Pro | Ile | Ser | Leu | Gly | Gly | Phe | Leu | Ser | Gly |      |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |      |
| 15 | ctg | gcc | gga | cgt | ctg | ctg | aca | gcg | ttg | ggg | gtg | gat | cag | tcg | cag | gcc | 672  |
|    | Leu | Ala | Gly | Arg | Leu | Leu | Thr | Ala | Leu | Gly | Val | Asp | Gln | Ser | Gln | Ala |      |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |      |
| 20 | gac | caa | att | gcc | aag | atc | gtc | gcc | ggc | ctg | gcc | gtg | ccc | gcc | gtc | ttg | 720  |
|    | Asp | Gln | Ile | Ala | Lys | Ile | Val | Ala | Gly | Leu | Ala | Val | Pro | Ala | Val | Leu |      |
|    | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |      |
| 25 | ctg | atc | gaa | ccc | cag | atg | ctg | ggc | gaa | atg | gcc | gaa | ggc | gtg | gcc | agg | 768  |
|    | Leu | Ile | Glu | Pro | Gln | Met | Leu | Gly | Glu | Met | ala | Glu | Gly | Val | Ala | Arg |      |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |      |
| 30 | ctg | gcg | ggc | gcc | ggc | gat | gcc | acc | gcg | gga | tac | ata | gcc | atg | gcg | atg | 816  |
|    | Leu | Ala | Gly | Ala | Gly | Asp | Ala | Thr | Ala | Gly | Tyr | Ile | Ala | Met | ala | Met |      |
|    |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |      |
| 35 | tcc | atc | gtg | gcg | gcg | atc | gcg | gtc | gcc | gcg | atc | aat | gcc | gcc | ggg | acg | 864  |
|    | Ser | Ile | Val | Ala | Ala | Ile | Ala | Val | Ala | Ala | Ile | Asn | Ala | Ala | Gly | Thr |      |
|    |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |      |
| 40 | gcc | ggc | gcg | ggc | agc | gcc | tcg | gcg | atc | agg | ggg | gcc | tcg | gat | cgg | gcc | 912  |
|    | Ala | Gly | Ala | Gly | Ser | Ala | Ser | Ala | Ile | Arg | Gly | Ala | Trp | Asp | Arg | Ala |      |
|    |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |      |
| 45 | gcc | gcg | gta | gcc | acc | cag | gtc | ctt | cag | ggg | ggg | acg | gca | gtg | gcg | caa | 960  |
|    | Ala | Ala | Val | Ala | Thr | Gln | Val | Leu | Gln | Gly | Gly | Thr | Ala | Val | Ala | Gln |      |
|    |     | 305 |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |      |
| 50 | ggc | ggc | gtc | ggc | gtg | tcg | atg | gca | gtc | gat | cgc | aaa | cag | gcc | gat | ctc | 1008 |
|    | Gly | Gly | Val | Gly | Val | Ser | Met | ala | Val | Asp | Arg | Lys | Gln | Ala | Asp | Leu |      |
|    |     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |      |
| 55 | ctg | gtc | gcc | gac | aag | gcg | gat | ctg | gcg | gcg | agc | ctg | aca | aaa | ctg | cgg | 1056 |
|    | Leu | Val | Ala | Asp | Lys | Ala | Asp | Leu | Ala | Ala | Ser | Leu | Thr | Lys | Leu | Arg |      |
|    |     |     |     | 340 |     |     |     | 345 |     |     |     |     |     | 350 |     |     |      |
| 60 | gcg | gcc | atg | gag | cgt | gag | gcg | gac | gat | atc | aag | aag | atc | ctg | gct | caa | 1104 |
|    | Ala | Ala | Met | Glu | Arg | Glu | Ala | Asp | Asp | Ile | Lys | Lys | Ile | Leu | Ala | Gln |      |
|    |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |      |
| 65 | ttc | gac | gcg | gcc | tat | cac | atg | atc | gcg | cag | atg | atc | agc | gac | atg | gcg | 1152 |
|    | Phe | Asp | Ala | Ala | Tyr | His | Met | Ile | Ala | Gln | Met | Ile | Ser | Asp | Met | ala |      |
|    |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |      |
| 70 | agc | acg | cac | agc | cag | gtc | agc | gcc | aac | ctc | gga | cgg | cgc | cag | gcg | gtg | 1200 |
|    | Ser | Thr | His | Ser | Gln | Val | Ser | Ala | Asn | Leu | Gly | Arg | Arg | Gln | Ala | Val |      |
|    |     | 385 |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |      |
| 75 | tag |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 1203 |

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 <212> PRT  
 <213> Bordetella pertussis

<400> 58

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
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| 10 | Met | Thr | Val | Met | Ser | Thr | Thr | Ile | Ser | Thr | Ala | Pro | Ser | Gly | Ala | Ala |
|    | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |     |
|    | Leu | Ala | Pro | Ser | Arg | Ile | Asp | Met | Arg | Ala | Pro | Glu | Pro | Gly | Ser | Ala |
|    |     |     |     | 20  |     |     |     | 25  |     |     |     |     |     | 30  |     |     |
|    | Gly | Glu | Gly | Ala | Gly | Ile | Leu | Ala | Pro | Val | Thr | Thr | Leu | Ala | Leu | Ala |
|    |     |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |
| 15 | Ala | Gly | Arg | Pro | Ala | Leu | Pro | Ala | Ser | Pro | Ser | Leu | Arg | Thr | Ala | Pro |
|    | 50  |     |     |     |     | 55  |     |     |     |     |     | 60  |     |     |     |     |
|    | Val | Leu | Asp | Pro | Pro | Val | Arg | Asp | Leu | Ser | Pro | Ala | Asp | Leu | Ala | Asp |
|    | 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     | 80  |     |     |
|    | Leu | Leu | Arg | Val | Leu | Arg | Ser | Arg | Ala | Val | Asp | Gly | Gln | Leu | Ala | Thr |
|    |     |     |     | 85  |     |     |     |     |     | 90  |     |     |     | 95  |     |     |
| 20 | Ala | Arg | Glu | Asn | Leu | Gln | Asp | Ala | Gln | Val | Lys | Ala | Lys | Gln | Asn | Thr |
|    |     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
|    | Gln | Ala | Gln | Leu | Asp | Lys | Leu | Asp | Ala | Trp | Phe | Arg | Lys | Ala | Glu | Asp |
|    |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| 25 | Ala | Glu | Ser | Lys | Gly | Trp | Leu | Ser | Lys | Val | Phe | Gly | Trp | Ile | Gly | Lys |
|    | 130 |     |     |     |     | 135 |     |     |     |     |     | 140 |     |     |     |     |
|    | Val | Leu | Ala | Val | Val | Ala | Ser | Ala | Leu | Ala | Val | Gly | Phe | Ala | Ala | Val |
|    | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     | 160 |     |
|    | Ala | Ser | Val | Val | Thr | Gly | Ala | Ala | Ala | Thr | Pro | Met | Leu | Val | Leu | Ser |
|    |     |     |     | 165 |     |     |     |     |     | 170 |     |     |     | 175 |     |     |
| 30 | Gly | Met | Ala | Leu | Val | Ser | Ala | Val | Thr | Ser | Leu | Ala | Asp | Gln | Ile | Ser |
|    |     |     | 180 |     |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
|    | Arg | Glu | Ala | Gly | Gly | Pro | Pro | Ile | Ser | Leu | Gly | Gly | Phe | Leu | Ser | Gly |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| 35 | Leu | Ala | Gly | Arg | Leu | Leu | Thr | Ala | Leu | Gly | Val | Asp | Gln | Ser | Gln | Ala |
|    | 210 |     |     |     |     | 215 |     |     |     |     |     | 220 |     |     |     |     |
|    | Asp | Gln | Ile | Ala | Lys | Ile | Val | Ala | Gly | Leu | Ala | Val | Pro | Ala | Val | Leu |
|    | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     | 240 |     |
|    | Leu | Ile | Glu | Pro | Gln | Met | Leu | Gly | Glu | Met | Ala | Glu | Gly | Val | Ala | Arg |
|    |     |     |     | 245 |     |     |     |     |     | 250 |     |     |     | 255 |     |     |
| 40 | Leu | Ala | Gly | Ala | Gly | Asp | Ala | Thr | Ala | Gly | Tyr | Ile | Ala | Met | Ala | Met |
|    |     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
|    | Ser | Ile | Val | Ala | Ala | Ile | Ala | Val | Ala | Ala | Ile | Asn | Ala | Ala | Gly | Thr |
|    |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| 45 | Ala | Gly | Ala | Gly | Ser | Ala | Ser | Ala | Ile | Arg | Gly | Ala | Trp | Asp | Arg | Ala |
|    | 290 |     |     |     |     | 295 |     |     |     |     |     | 300 |     |     |     |     |
|    | Ala | Ala | Val | Ala | Thr | Gln | Val | Leu | Gln | Gly | Gly | Thr | Ala | Val | Ala | Gln |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     | 320 |     |
|    | Gly | Gly | Val | Gly | Val | Ser | Met | Ala | Val | Asp | Arg | Lys | Gln | Ala | Asp | Leu |
|    |     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |
| 50 | Leu | Val | Ala | Asp | Lys | Ala | Asp | Leu | Ala | Ala | Ser | Leu | Thr | Lys | Leu | Arg |
|    |     |     | 340 |     |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
|    | Ala | Ala | Met | Glu | Arg | Glu | Ala | Asp | Asp | Ile | Lys | Lys | Ile | Leu | Ala | Gln |
|    |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| 55 | Phe | Asp | Ala | Ala | Tyr | His | Met | Ile | Ala | Gln | Met | Ile | Ser | Asp | Met | Ala |
|    | 370 |     |     |     |     | 375 |     |     |     |     |     | 380 |     |     |     |     |
|    | Ser | Thr | His | Ser | Gln | Val | Ser | Ala | Asn | Leu | Gly | Arg | Arg | Gln | Ala | Val |
|    | 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     | 400 |     |

60 <210> 59  
 <211> 462







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|    |   |  |     |  |     |     |     |
|----|---|--|-----|--|-----|-----|-----|
|    | 130   |  | 135 |  | 140 |     |     |
| 5  | agg ctg gcg cag gtc gtg ttg gac agc gga ccc gcc atg atg cgg ccc |  |     |  |     | 480 |     |
|    | Arg Leu Ala Gln Val Val Leu Asp Ser Gly Pro Ala Met Met Arg Pro |  |     |  |     |     |     |
|    | 145   |  | 150 |  | 155 |     | 160 |
| 10 | gcg ccg ttg cag tcc gag cca tta cct gga gct cct gga tga         |  |     |  |     | 522 |     |
|    | Ala Pro Leu Gln Ser Glu Pro Leu Pro Gly Ala Pro Gly *           |  |     |  |     |     |     |
|    |   |  | 165 |  | 170 |     |     |
| 15 | <210> 62  |  |     |  |     |     |     |
|    | <211> 173   |  |     |  |     |     |     |
|    | <212> PRT   |  |     |  |     |     |     |
|    | <213> Bordetella pertussis                                      |  |     |  |     |     |     |
| 20 | <400> 62  |  |     |  |     |     |     |
|    | Met His Ser Asp Ser Gly Ser Asp Ser Gly Ser Asp Ser Gly Ser Gly |  |     |  |     |     |     |
|    | 1 5 10 15   |  |     |  |     |     |     |
|    | Ser Pro Met Val Ser Ser Ile His Pro Ser Glu Pro Ile Gln Pro Met |  |     |  |     |     |     |
|    | 20 25 30  |  |     |  |     |     |     |
|    | Glu His Val Leu Glu Glu Ala Asp Ala Arg Leu Leu Thr Glu Val Gly |  |     |  |     |     |     |
|    | 35 40 45  |  |     |  |     |     |     |
| 25 | Phe Leu Ala Ala Ala Val Ser Asp Leu Thr Arg Ala Asp Ala Ile Phe |  |     |  |     |     |     |
|    | 50 55 60  |  |     |  |     |     |     |
|    | Asn Ala Leu Gln Arg Val Arg Pro Gly Arg Thr His Pro Cys Ile Gly |  |     |  |     |     |     |
|    | 65 70 75 80   |  |     |  |     |     |     |
|    | Leu Ala Val Ala Arg Met Asn Ala Gly Leu Pro Asp Glu Ala Ala Glu |  |     |  |     |     |     |
|    | 85 90 95  |  |     |  |     |     |     |
| 30 | Ile Leu Ala Asn Phe Gln Pro Ala Gln Pro Glu Asp Arg Ser Glu Leu |  |     |  |     |     |     |
|    | 100 105 110   |  |     |  |     |     |     |
|    | Asp Ala Trp Cys Gly Phe Ala Leu Leu Ala Gly Arg Ser Asp Glu     |  |     |  |     |     |     |
|    | 115 120 125   |  |     |  |     |     |     |
| 35 | Ala Arg Arg Met Leu Gln Arg Ala Ile Asp Ala Gly Gly Glu Ala Ala |  |     |  |     |     |     |
|    | 130 135 140   |  |     |  |     |     |     |
|    | Arg Leu Ala Gln Val Val Leu Asp Ser Gly Pro Ala Met Met Arg Pro |  |     |  |     |     |     |
|    | 145 150 155 160   |  |     |  |     |     |     |
|    | Ala Pro Leu Gln Ser Glu Pro Leu Pro Gly Ala Pro Gly             |  |     |  |     |     |     |
|    |   |  | 165 |  | 170 |     |     |
| 40 | <210> 63  |  |     |  |     |     |     |
|    | <211> 180   |  |     |  |     |     |     |
|    | <212> DNA   |  |     |  |     |     |     |
|    | <213> Bordetella pertussis                                      |  |     |  |     |     |     |
| 45 | <220>   |  |     |  |     |     |     |
|    | <221> CDS   |  |     |  |     |     |     |
|    | <222> (1)...(180)   |  |     |  |     |     |     |
| 50 | <400> 63  |  |     |  |     |     |     |
|    | atg gtt gcg cga ggt cct tgc gca cca cct ggt gta gtc ccc ata cgt |  |     |  |     | 48  |     |
|    | Met Val Ala Arg Gly Pro Cys Ala Pro Pro Gly Val Val Pro Ile Arg |  |     |  |     |     |     |
|    | 1 5 10 15   |  |     |  |     |     |     |
| 55 | cgc gtc gta agt ttt acg gaa gac gtt cag cgc gtt gta tct gaa tgc |  |     |  |     | 96  |     |
|    | Arg Val Val Ser Phe Thr Glu Asp Val Gln Arg Val Val Ser Glu Cys |  |     |  |     |     |     |
|    | 20 25 30  |  |     |  |     |     |     |
| 60 | tcc ggt agc gac cgc gat ccg aca tta gtt tcg gaa gtt aac aac tgc |  |     |  |     | 144 |     |
|    | Ser Gly Ser Asp Arg Asp Pro Thr Leu Val Ser Glu Val Asn Asn Cys |  |     |  |     |     |     |

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                35                40                45
cgg ata aaa ccg gca gtc agt atc ttg gtg acg tga
Arg Ile Lys Pro Ala Val Ser Ile Leu Val Thr *
5      50                        55                                180

    <210> 64
    <211> 59
10    <212> PRT
    <213> Bordetella pertussis

    <400> 64
15    Met Val Ala Arg Gly Pro Cys Ala Pro Pro Gly Val Val Pro Ile Arg
      1      5      10      15
      Arg Val Val Ser Phe Thr Glu Asp Val Gln Arg Val Val Ser Glu Cys
      20      25      30
      Ser Gly Ser Asp Arg Asp Pro Thr Leu Val Ser Glu Val Asn Asn Cys
      35      40      45
20    Arg Ile Lys Pro Ala Val Ser Ile Leu Val Thr
      50                        55

    <210> 65
    <211> 975
25    <212> DNA
    <213> Bordetella pertussis

    <220>
    <221> CDS
30    <222> (1)...(975)

    <400> 65
35    atg cgg ttt cga gca ggt tat agc cgt tat caa gcc cgc tca ggc cat
      Met Arg Phe Arg Ala Gly Tyr Ser Arg Tyr Gln Ala Arg Ser Gly His
      1      5      10      15                                48

      ggg gac cgc cca ccc ccc gca cag gcg cgc gtc cag acg gta ctc ctg
      Gly Asp Arg Pro Pro Pro Ala Gln Ala Arg Val Gln Thr Val Leu Leu
      20      25      30                                96

40    cac gga ctc tcc gcg ttg acg gcg caa gtt gcg cag cgc ttc gaa atg
      His Gly Leu Ser Ala Leu Thr Ala Gln Val Ala Gln Arg Phe Glu Met
      35      40      45                                144

45    gcg cgc cac cgg atg gct ggc ccc ggt cgc acg aca ggc cac cac cat
      Ala Arg His Arg Met ala Gly Pro Gly Arg Thr Thr Gly His His His
      50      55      60                                192

50    ttc cag ctc gag gcc cag cgt atg gcc gac act ttg cgc agc gtt caa
      Phe Gln Leu Glu Ala Gln Arg Met ala Asp Thr Leu Arg Ser Val Gln
      65      70      75      80                                240

      ggc gag cct cgg tgg ccg gac ggg agc gag gcc tgc atg ccg tgc ggt
      Gly Glu Pro Arg Trp Pro Asp Gly Ser Glu Ala Cys Met Pro Ser Gly
      85      90      95                                288

      ttg tca tgc cgg cat gga acc gaa gag ccg aaa gcg tca cac agt gca
      Leu Ser Cys Arg His Gly Thr Glu Glu Pro Lys Ala Ser His Ser Ala
      100      105      110                                336
60

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<213> Bordetella pertussis

<400> 66

5 Met Arg Phe Arg Ala Gly Tyr Ser Arg Tyr Gln Ala Arg Ser Gly His  
1 5 10 15  
Gly Asp Arg Pro Pro Ala Gln Ala Arg Val Gln Thr Val Leu Leu  
20 25 30  
His Gly Leu Ser Ala Leu Thr Ala Gln Val Ala Gln Arg Phe Glu Met  
35 40 45  
10 Ala Arg His Arg Met ala Gly Pro Gly Arg Thr Thr Gly His His His  
50 55 60  
Phe Gln Leu Glu Ala Gln Arg Met ala Asp Thr Leu Arg Ser Val Gln  
65 70 75 80  
15 Gly Glu Pro Arg Trp Pro Asp Gly Ser Glu Ala Cys Met Pro Ser Gly  
85 90 95  
Leu Ser Cys Arg His Gly Thr Glu Glu Pro Lys Ala Ser His Ser Ala  
100 105 110  
Tyr Ser Met Phe Pro Leu Arg Arg Thr Arg Tyr Thr Gln Gly Phe Glu  
115 120 125  
20 Thr Thr Ala His Arg Met Asn Phe Gln Ile Pro Pro Ala Leu Pro Ala  
130 135 140  
Leu Glu Leu Asp Val Phe Ala Arg Ala Ala Ser Gln Gly Glu Thr Leu  
145 150 155 160  
25 Tyr Val Thr Lys Ala Gly Glu Gln Phe Gln Val Ile Ala Ser Gly Thr  
165 170 175  
Thr Pro Ser Gly Arg Asn Val Ser Trp Val Ala Thr Asp Glu Asp Thr  
180 185 190  
Leu Val Met Phe Ser Ser Ala Leu Ala Leu Ala Tyr Gly Thr Gly Ile  
195 200 205  
30 Ala Arg Ala Val Ala Lys Glu Leu Asp Leu His Ala Val Pro Thr Thr  
210 215 220  
Ser Leu Ser Ala Arg Val Val Thr Arg Ala Val Asp Met ala Glu Thr  
225 230 235 240  
35 Ser Arg His Ala Leu Gln Gly Val Asp Phe Leu Thr Phe Leu Ser Trp  
245 250 255  
Ser Ala Arg Ala Asp Ala Ala Gly Phe Arg Gln Val Cys His Asp Thr  
260 265 270  
Gly Val Ser Pro Asp Gln Ile Ser Gly Thr Leu Arg Ala Thr Ile Asp  
275 280 285  
40 Glu Ser Met Gln Gln Arg Phe Ala Ser Ala Ala Gln Ser Gly Lys Ala  
290 295 300  
Pro Val Ser Ala His Thr Ala Gln Glu Trp Leu Arg Glu Val Leu Ala  
305 310 315 320  
45 His His Leu Val

<210> 67

<211> 1146

<212> DNA

50 <213> Bordetella pertussis

<220>

<221> CDS

55 <222> (1)...(1146)

<400> 67

atg ctg atc aac gcg gcc gag cac ccc gcc gcc agc ctg gat gcc gac  
Met Leu Ile Asn Ala Ala Glu His Pro Ala Ala Ser Leu Asp Ala Asp  
1 5 10 15  
60

48



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|    |   |      |
|----|---|------|
|    | ctg agc tca gcc ggc gat gca gtc gcg gcc agt gcg ccg atg gcg gcg | 816  |
|    | Leu Ser Ser Ala Gly Asp Ala Val Ala Ala Ser Ala Pro Met ala Ala |      |
|    | 260 265 270   |      |
| 5  | tcg gcg ctg caa ttg ggg ctg ttg cgc aat ctg cac gac gcc gag gcc | 864  |
|    | Ser Ala Leu Gln Leu Gly Leu Leu Arg Asn Leu His Asp Ala Glu Ala |      |
|    | 275 280 285   |      |
| 10 | ctg gtg agg cga tgg ggc tgg ctg cgc ttg cgt gcg ttg cgc gat cgg | 912  |
|    | Leu Val Arg Arg Trp Gly Trp Leu Arg Leu Arg Ala Leu Arg Asp Arg |      |
|    | 290 295 300   |      |
| 15 | gcc atc gct ttg gcg ttg gac gat gcg cag gtg cgc tgc ctt tgc caa | 960  |
|    | Ala Ile Ala Leu Ala Leu Asp Asp Ala Gln Val Arg Cys Leu Cys Gln |      |
|    | 305 310 315 320   |      |
| 20 | cag gtc gtg gcg gta gcc gaa ggc ggg ctg gcc gcc gac gag cag caa | 1008 |
|    | Gln Val Val Ala Val Ala Glu Gly Gly Leu Ala Gly Asp Glu Gln Gln |      |
|    | 325 330 335   |      |
| 25 | tgg ctc gat tat gtg cgt tac gtg gtg gaa acc gcc gag acc gcc gcg | 1056 |
|    | Trp Leu Asp Tyr Val Arg Tyr Val Val Glu Thr Gly Glu Thr Ala Ala |      |
|    | 340 345 350   |      |
| 30 | gac cgc atg ctg cgc ttg tgg cgc cag gcg cgc gcc acg cct gag atg | 1104 |
|    | Asp Arg Met Leu Arg Leu Trp Arg Gln Ala Arg Gly Thr Pro Glu Met |      |
|    | 355 360 365   |      |
| 35 | cgc cgc gca cag gcg tgc cgg cag cgc gcg gtg ctg tcc tag         | 1146 |
|    | Arg Arg Ala Gln Ala Cys Arg Gln Arg Ala Val Leu Ser *           |      |
|    | 370 375 380   |      |
| 35 | <210> 68  |      |
|    | <211> 381   |      |
|    | <212> PRT   |      |
|    | <213> Bordetella pertussis                                      |      |
| 40 | <400> 68  |      |
|    | Met Leu Ile Asn Ala Ala Glu His Pro Ala Ala Ser Leu Asp Ala Asp |      |
|    | 1 5 10 15   |      |
|    | Trp Tyr Arg Arg Val Arg Val Pro Arg Pro Ile Tyr Glu Glu Leu Val |      |
|    | 20 25 30  |      |
| 45 | Gly Gln Arg Gly Trp Leu His Arg Ile Gly Ile Asp Ala Lys Ala Gln |      |
|    | 35 40 45  |      |
|    | Asn Ser Pro Cys Thr Ser Val Pro Val Ala Ile Ala Ala Arg Cys Leu |      |
|    | 50 55 60  |      |
|    | Asn Val Val Leu Ala Leu Ala Pro Ala Gln Ile Ala Met Phe Ala Asn |      |
|    | 65 70 75 80   |      |
| 50 | Ser Pro Leu Glu Ala Gly Arg Val Thr Gly Leu Lys Glu Asn Arg Leu |      |
|    | 85 90 95  |      |
|    | Thr Leu Trp Pro Arg Met Phe Arg Gly Ala Arg Tyr Leu Gly Asp Asp |      |
|    | 100 105 110   |      |
| 55 | Leu Leu His Arg Leu Pro Ala Arg Pro Phe Arg Asp Leu Gly Asp Tyr |      |
|    | 115 120 125   |      |
|    | Phe Arg Trp Met Phe Gly Gly Leu Thr Ala Ser Arg Ala Leu Pro Pro |      |
|    | 130 135 140   |      |
|    | Gly Asp Ala Cys Asp Tyr Lys Asn Ala Asp Val Ala Cys Leu Val Gly |      |
|    | 145 150 155 160   |      |
| 60 | Ala Pro Ser Leu Ala Glu Phe Leu Tyr Ala Gly Ala Trp Ser Ala Arg |      |

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|    |     |     |     |       |                      |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|-----|-----|-----|-------|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    |     |     |     |       | 165                  |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     |
|    | Asn | Leu | Asn | Asp   | Gly                  | Gly | Ser | Val | Arg | Leu | Ala | Ala | Arg | Ser | Glu | His |     |     |
|    |     |     |     | 180   |                      |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     |
| 5  | Phe | Val | Tyr | Ser   | Gln                  | Phe | Ala | Gln | Phe | Leu | Asp | Ala | Arg | Trp | Arg | Tyr |     |     |
|    |     |     | 195 |       |                      |     |     | 200 |     |     |     |     | 205 |     |     |     |     |     |
|    | Arg | Met | Pro | Ile   | Val                  | Pro | Ala | Leu | Pro | Ala | Leu | Leu | Arg | Ala | Trp | Asp |     |     |
|    |     | 210 |     |       |                      |     | 215 |     |     |     |     | 220 |     |     |     |     |     |     |
|    | Arg | Gln | Gly | Gly   | Leu                  | Glu | Ala | Leu | Phe | Glu | Gln | Ala | Gly | Ala | Gln | Gly |     |     |
| 10 |     | 225 |     |       | 230                  |     |     |     |     |     | 235 |     |     |     |     | 240 |     |     |
|    | Tyr | Ile | Glu | Gly   | Arg                  | Ala | Pro | Gly | Ala | Val | Phe | Ala | Asp | Ala | Asp | Leu |     |     |
|    |     |     |     | 245   |                      |     |     |     |     | 250 |     |     |     |     | 255 |     |     |     |
|    | Leu | Ser | Ser | Ala   | Gly                  | Asp | Ala | Val | Ala | Ala | Ser | Ala | Pro | Met | ala | Ala |     |     |
|    |     |     |     | 260   |                      |     |     |     | 265 |     |     |     |     | 270 |     |     |     |     |
| 15 | Ser | Ala | Leu | Gln   | Leu                  | Gly | Leu | Leu | Arg | Asn | Leu | His | Asp | Ala | Glu | Ala |     |     |
|    |     |     | 275 |       |                      |     |     | 280 |     |     |     |     | 285 |     |     |     |     |     |
|    | Leu | Val | Arg | Arg   | Trp                  | Gly | Trp | Leu | Arg | Leu | Arg | Ala | Leu | Arg | Asp | Arg |     |     |
|    |     | 290 |     |       |                      |     | 295 |     |     |     |     | 300 |     |     |     |     |     |     |
|    | Ala | Ile | Ala | Leu   | Ala                  | Leu | Asp | Asp | Ala | Gln | Val | Arg | Cys | Leu | Cys | Gln |     |     |
| 20 |     | 305 |     |       | 310                  |     |     |     |     |     | 315 |     |     |     |     | 320 |     |     |
|    | Gln | Val | Val | Ala   | Val                  | Ala | Glu | Gly | Gly | Leu | Ala | Gly | Asp | Glu | Gln | Gln |     |     |
|    |     |     |     | 325   |                      |     |     |     |     | 330 |     |     |     |     | 335 |     |     |     |
|    | Trp | Leu | Asp | Tyr   | Val                  | Arg | Tyr | Val | Val | Glu | Thr | Gly | Glu | Thr | Ala | Ala |     |     |
|    |     |     |     | 340   |                      |     |     |     | 345 |     |     |     |     | 350 |     |     |     |     |
| 25 | Asp | Arg | Met | Leu   | Arg                  | Leu | Trp | Arg | Gln | Ala | Arg | Gly | Thr | Pro | Glu | Met |     |     |
|    |     |     | 355 |       |                      |     | 360 |     |     |     |     |     | 365 |     |     |     |     |     |
|    | Arg | Arg | Ala | Gln   | Ala                  | Cys | Arg | Gln | Arg | Ala | Val | Leu | Ser |     |     |     |     |     |
|    |     | 370 |     |       |                      |     | 375 |     |     |     |     | 380 |     |     |     |     |     |     |
| 30 |     |     |     | <210> | 69                   |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    |     |     |     | <211> | 1233                 |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    |     |     |     | <212> | DNA                  |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    |     |     |     | <213> | Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 35 |     |     |     | <220> |                      |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    |     |     |     | <221> | CDS                  |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    |     |     |     | <222> | (1)...(1233)         |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 40 |     |     |     | <400> | 69                   |     |     |     |     |     |     |     |     |     |     |     |     |     |
|    | atg | acc | ctt | cgc   | ccc                  | gga | ata | ctc | gcc | ccg | ctc | gcc | ctg | ctt | ctt | ggc | 48  |     |
|    | Met | Thr | Leu | Arg   | Pro                  | Gly | Ile | Leu | Ala | Pro | Leu | Ala | Leu | Leu | Leu | Gly |     |     |
|    | 1   |     |     |       | 5                    |     |     |     |     | 10  |     |     |     |     | 15  |     |     |     |
| 45 |     |     |     | ctc   | gcg                  | tgg | ccc | atc | gcg | gcg | gca | tcc | agc | cct | cct | cct | gtc | 96  |
|    | Leu | Ala | Trp | Pro   | Ile                  | Ala | Ala | Ala | Ser | Ser | Pro | Pro | Pro | Pro | Val | Ala | Ala |     |
|    |     |     |     | 20    |                      |     |     |     | 25  |     |     |     |     |     | 30  |     |     |     |
| 50 |     |     |     | ctg   | agc                  | tcc | ggc | gtc | gcc | ctt | acc | tgc | ccc | cgc | ctt | ccg | cct | 144 |
|    | Leu | Ser | Ser | Gly   | Val                  | Ala | Leu | Thr | Ser | Pro | Arg | Leu | Pro | Pro | Pro | Ser |     |     |
|    |     |     |     | 35    |                      |     |     | 40  |     |     |     |     | 45  |     |     |     |     |     |
| 55 |     |     |     | cat   | aca                  | tcc | ggc | cgg | aaa | tgg | cgc | atc | ggt | tat | gtg | ggt | agc | 192 |
|    | His | Thr | Ser | Gly   | Arg                  | Lys | Trp | Arg | Ile | Gly | Tyr | Val | Gly | Ser | Gly | Glu |     |     |
|    |     | 50  |     |       |                      |     | 55  |     |     |     |     | 60  |     |     |     |     |     |     |
| 60 |     |     |     | tac   | gag                  | gag | tat | ccg | cgc | acg | ctc | tac | gcg | atc | gcg | cgc | gca | 240 |
|    | Tyr | Glu | Glu | Tyr   | Pro                  | Arg | Thr | Leu | Tyr | Ala | Ile | Ala | Arg | Ala | Leu | Gln |     |     |
|    |     | 65  |     |       |                      |     | 70  |     |     |     | 75  |     |     |     |     | 80  |     |     |
| 60 |     |     |     | caa   | ctc                  | gga | tgg | ctg | cgt | atc | gac | gac | atg | ccc | gag | ata | acc | 288 |
|    | Gln | Leu | Gly | Trp   | Leu                  | Arg | Ile | Asp | Asp | Met | Pro | Glu | Ile | Thr | Asp | Met |     |     |

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|    | 85  |     |     |     |     |     |     |     |     |     | 90  |     |     |     |     | 95  |      |  |  |  |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|--|--|--|--|
| 5  | cga | aag | gcc | tgg | ctt | tac | ctg | gcc | acg | cat | gcc | cgc | agc | aac | tac | atc | 336  |  |  |  |  |
|    | Arg | Lys | Ala | Trp | Leu | Tyr | Leu | Ala | Thr | His | Ala | Arg | Ser | Asn | Tyr | Ile |      |  |  |  |  |
|    |     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |      |  |  |  |  |
| 10 | gag | ttc | gtg | ccc | gat | gcg | tgg | tgg | cag | ccc | ggc | aac | ttc | gac | acc | gcc | 384  |  |  |  |  |
|    | Glu | Phe | Val | Pro | Asp | Ala | Trp | Trp | Gln | Pro | Gly | Asn | Phe | Asp | Thr | Ala |      |  |  |  |  |
|    |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |      |  |  |  |  |
| 15 | ttg | cgg | cct | gcc | gtg | cgc | gaa | gcc | gtt | gcg | gca | cgc | ctg | cat | ggc | gcc | 432  |  |  |  |  |
|    | Leu | Arg | Pro | Ala | Val | Arg | Glu | Ala | Val | Ala | Ala | Arg | Leu | His | Gly | Ala |      |  |  |  |  |
|    |     |     | 130 |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |      |  |  |  |  |
| 20 | aag | gac | atc | gac | ctg | atc | atc | gcc | atg | ggt | acc | tgg | gct | gga | cag | gac | 480  |  |  |  |  |
|    | Lys | Asp | Ile | Asp | Leu | Ile | Ile | Ala | Met | Gly | Thr | Trp | Ala | Gly | Gln | Asp |      |  |  |  |  |
|    |     |     | 145 |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |      |  |  |  |  |
| 25 | atg | gtc | gaa | ctg | ggc | acg | ccg | gta | ccc | acc | gtg | gtc | gtc | tcg | tcg | acc | 528  |  |  |  |  |
|    | Met | Val | Glu | Leu | Gly | Thr | Pro | Val | Pro | Thr | Val | Val | Val | Ser | Ser | Thr |      |  |  |  |  |
|    |     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |      |  |  |  |  |
| 30 | gac | ccg | ata | agc | gcc | cgg | atc | ata | ccc | agt | gcg | gcc | gac | agc | ggc | cag | 576  |  |  |  |  |
|    | Asp | Pro | Ile | Ser | Ala | Arg | Ile | Ile | Pro | Ser | Ala | Ala | Asp | Ser | Gly | Gln |      |  |  |  |  |
|    |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |      |  |  |  |  |
| 35 | gac | aac | ctg | cat | gcc | cgg | gta | cag | ccc | gac | cac | tac | cag | cgg | cag | atc | 624  |  |  |  |  |
|    | Asp | Asn | Leu | His | Ala | Arg | Val | Gln | Pro | Asp | His | Tyr | Gln | Arg | Gln | Ile |      |  |  |  |  |
|    |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |      |  |  |  |  |
| 40 | cag | ctg | ctc | cat | gac | atc | gtg | ccg | ttc | aag | acg | ctt | gga | ctg | gtc | tac | 672  |  |  |  |  |
|    | Gln | Leu | Leu | His | Asp | Ile | Val | Pro | Phe | Lys | Thr | Leu | Gly | Leu | Val | Tyr |      |  |  |  |  |
|    |     |     | 210 |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |      |  |  |  |  |
| 45 | gaa | gac | acc | gaa | gca | ggt | cgc | acc | tac | gca | gcc | atc | gat | aag | gtc | gcc | 720  |  |  |  |  |
|    | Glu | Asp | Thr | Glu | Ala | Gly | Arg | Thr | Tyr | Ala | Ala | Ile | Asp | Lys | Val | Ala |      |  |  |  |  |
|    |     |     | 225 |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |      |  |  |  |  |
| 50 | gca | cta | atg | ccg | gca | ttg | gat | ttc | tcc | gtc | aag | cgt | tgc | gac | gca | cgc | 768  |  |  |  |  |
|    | Ala | Leu | Met | Pro | Ala | Leu | Asp | Phe | Ser | Val | Lys | Arg | Cys | Asp | Ala | Arg |      |  |  |  |  |
|    |     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |      |  |  |  |  |
| 55 | gcg | acc | ggc | atc | ccc | atc | gcc | acg | gca | acc | cag | aac | gtt | ctg | gct | tgc | 816  |  |  |  |  |
|    | Ala | Thr | Gly | Ile | Pro | Ile | Ala | Thr | Ala | Thr | Gln | Asn | Val | Leu | Ala | Cys |      |  |  |  |  |
|    |     |     |     | 260 |     |     |     | 265 |     |     |     |     |     | 270 |     |     |      |  |  |  |  |
| 60 | tac | cag | aag | ctg | tcg | agc | gaa | gtc | gac | gcc | ttt | tac | gtc | acc | gag | cac | 864  |  |  |  |  |
|    | Tyr | Gln | Lys | Leu | Ser | Ser | Glu | Val | Asp | Ala | Phe | Tyr | Val | Thr | Glu | His |      |  |  |  |  |
|    |     |     | 275 |     |     |     | 280 |     |     |     |     |     | 285 |     |     |     |      |  |  |  |  |
| 65 | cgg | ggc | atc | acc | tcg | acg | tcc | gtc | aag | cag | ctc | gcc | gcg | ctg | ctg | cgc | 912  |  |  |  |  |
|    | Arg | Gly | Ile | Thr | Ser | Thr | Ser | Val | Lys | Gln | Leu | Ala | Ala | Leu | Leu | Arg |      |  |  |  |  |
|    |     |     | 290 |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |      |  |  |  |  |
| 70 | gcc | gcc | cgc | gtg | ccg | agt | ttc | tcg | atg | caa | ggc | tcc | gac | gag | gtc | aag | 960  |  |  |  |  |
|    | Ala | Ala | Arg | Val | Pro | Ser | Phe | Ser | Met | Gln | Gly | Ser | Asp | Glu | Val | Lys |      |  |  |  |  |
|    |     |     | 305 |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |      |  |  |  |  |
| 75 | gcc | ggc | ctg | ttg | atg | agc | ctg | gcc | aag | gcg | gac | tac | tcc | agc | gta | ggc | 1008 |  |  |  |  |
|    | Ala | Gly | Leu | Leu | Met | Ser | Leu | Ala | Lys | Ala | Asp | Tyr | Ser | Ser | Val | Gly |      |  |  |  |  |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |  |  |  |  |



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|    |       |                      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|----|-------|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
|    |       |                      |     | 325 |     |     |     |     | 330 |     |     |     | 335 |     |     |     |      |
| 5  | atg   | ttc                  | cac | gcc | cag | acc | att | gcc | cgc | att | ttc | aat | ggg | gaa | aag | ccg | 1056 |
|    | Met   | Phe                  | His | Ala | Gln | Thr | Ile | Ala | Arg | Ile | Phe | Asn | Gly | Glu | Lys | Pro |      |
|    |       |                      |     | 340 |     |     |     |     | 345 |     |     |     | 350 |     |     |     |      |
| 10 | cgc   | agc                  | atc | agc | cag | gtc | tgg | aat | gcc | ccc | gcc | aag | ata | gcc | atc | aat | 1104 |
|    | Arg   | Ser                  | Ile | Ser | Gln | Val | Trp | Asn | Ala | Pro | Ala | Lys | Ile | Ala | Ile | Asn |      |
|    |       |                      | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |      |
| 15 | ctg   | gaa                  | acg | gcg | cgg | cgc | atc | ggc | ttc | gac | cca | ccg | gtg | gat | att | ctg | 1152 |
|    | Leu   | Glu                  | Thr | Ala | Arg | Arg | Ile | Gly | Phe | Asp | Pro | Pro | Val | Asp | Ile | Leu |      |
|    |       | 370                  |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |      |
| 20 | ctg   | gcg                  | gcc | gac | gag | gtg | tac | gaa | gcg | gag | cac | tga | cag | gcc | tgg | cca | 1200 |
|    | Leu   | Ala                  | Ala | Asp | Glu | Val | Tyr | Glu | Ala | Glu | His | *   | Gln | Ala | Trp | Pro |      |
|    |       | 385                  |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     |     |      |
| 25 | acg   | aga                  | cct | ggc | aag | gaa | tgt | gcc | gga | tcc | tag |     |     |     |     |     | 1233 |
|    | Thr   | Arg                  | Pro | Gly | Lys | Glu | Cys | Ala | Gly | Ser | *   |     |     |     |     |     |      |
|    |       | 400                  |     |     |     | 405 |     |     |     |     |     |     |     |     |     |     |      |
| 30 | <210> | 70                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|    | <211> | 409                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|    | <212> | PRT                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|    | <213> | Bordetella pertussis |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
| 35 | <400> | 70                   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|    | Met   | Thr                  | Leu | Arg | Pro | Gly | Ile | Leu | Ala | Pro | Leu | Ala | Leu | Leu | Leu | Gly |      |
|    | 1     |                      |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |      |
|    | Leu   | Ala                  | Trp | Pro | Ile | Ala | Ala | Ala | Ser | Ser | Pro | Pro | Pro | Val | Ala | Ala |      |
|    |       |                      |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |      |
| 40 | Leu   | Ser                  | Ser | Gly | Val | Ala | Leu | Thr | Ser | Pro | Arg | Leu | Pro | Pro | Pro | Ser |      |
|    |       | 35                   |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |      |
|    | His   | Thr                  | Ser | Gly | Arg | Lys | Trp | Arg | Ile | Gly | Tyr | Val | Gly | Ser | Gly | Glu |      |
|    |       | 50                   |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |      |
|    | Tyr   | Glu                  | Glu | Tyr | Pro | Arg | Thr | Leu | Tyr | Ala | Ile | Ala | Arg | Ala | Leu | Gln |      |
|    |       | 65                   |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |      |
| 45 | Gln   | Leu                  | Gly | Trp | Leu | Arg | Ile | Asp | Asp | Met | Pro | Glu | Ile | Thr | Asp | Met |      |
|    |       |                      |     | 85  |     |     |     |     | 90  |     |     |     |     |     | 95  |     |      |
|    | Arg   | Lys                  | Ala | Trp | Leu | Tyr | Leu | Ala | Thr | His | Ala | Arg | Ser | Asn | Tyr | Ile |      |
|    |       |                      |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |      |
|    | Glu   | Phe                  | Val | Pro | Asp | Ala | Trp | Trp | Gln | Pro | Gly | Asn | Phe | Asp | Thr | Ala |      |
|    |       | 115                  |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |      |
| 50 | Leu   | Arg                  | Pro | Ala | Val | Arg | Glu | Ala | Val | Ala | Ala | Arg | Leu | His | Gly | Ala |      |
|    |       | 130                  |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |      |
|    | Lys   | Asp                  | Ile | Asp | Leu | Ile | Ile | Ala | Met | Gly | Thr | Trp | Ala | Gly | Gln | Asp |      |
|    |       | 145                  |     |     |     | 150 |     |     |     |     | 155 |     |     |     | 160 |     |      |
| 55 | Met   | Val                  | Glu | Leu | Gly | Thr | Pro | Val | Pro | Thr | Val | Val | Val | Ser | Ser | Thr |      |
|    |       |                      |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |      |
|    | Asp   | Pro                  | Ile | Ser | Ala | Arg | Ile | Ile | Pro | Ser | Ala | Ala | Asp | Ser | Gly | Gln |      |
|    |       |                      |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |      |
|    | Asp   | Asn                  | Leu | His | Ala | Arg | Val | Gln | Pro | Asp | His | Tyr | Gln | Arg | Gln | Ile |      |
|    |       | 195                  |     |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |      |
| 60 | Gln   | Leu                  | Leu | His | Asp | Ile | Val | Pro | Phe | Lys | Thr | Leu | Gly | Leu | Val | Tyr |      |
|    |       | 210                  |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |      |
|    | Glu   | Asp                  | Thr | Glu | Ala | Gly | Arg | Thr | Tyr | Ala | Ala | Ile | Asp | Lys | Val | Ala |      |
|    |       | 225                  |     |     |     | 230 |     |     |     |     | 235 |     |     |     | 240 |     |      |
|    | Ala   | Leu                  | Met | Pro | Ala | Leu | Asp | Phe | Ser | Val | Lys | Arg | Cys | Asp | Ala | Arg |      |



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|    |   |     |
|----|---|-----|
|    | cca ggc ctt tcg cat atc ggt tat ctc ggg cat gtc gtc gat acg cag   | 384 |
|    | Pro Gly Leu Ser His Ile Gly Tyr Leu Gly His Val Val Asp Thr Gln   |     |
|    | 115 120 125   |     |
| 5  | cca tcc gag ttg ttg caa tgc gcg cgc gat cgc gta gag cgt gcg cgg   | 432 |
|    | Pro Ser Glu Leu Leu Gln Cys Ala Arg Asp Arg Val Glu Arg Ala Arg   |     |
|    | 130 135 140   |     |
| 10 | ata ctc ctc gta ctc gcc gct acc cac ata acc gat gcg cca ttt ccg   | 480 |
|    | Ile Leu Leu Val Leu Ala Ala Thr His Ile Thr Asp Ala Pro Phe Pro   |     |
|    | 145 150 155 160   |     |
| 15 | gcc gga tgt atg gga ggg agg cgg aag gcg ggg cga ggt aag ggc gac   | 528 |
|    | Ala Gly Cys Met Gly Gly Arg Arg Lys Ala Gly Arg Gly Lys Gly Asp   |     |
|    | 165 170 175   |     |
| 20 | gcc gga gct cag ggc cgc gac agg agg agg gct gga tgc cgc cgc gat   | 576 |
|    | Ala Gly Ala Gln Gly Arg Asp Arg Arg Ala Gly Cys Arg Arg Asp   |     |
|    | 180 185 190   |     |
| 25 | ggg cca cgc gag gcc aag aag cag ggc gag cgg ggc gag tat tcc ggg   | 624 |
|    | Gly Pro Arg Glu Ala Lys Lys Gln Gly Glu Arg Gly Glu Tyr Ser Gly   |     |
|    | 195 200 205   |     |
| 30 | gcg aag ggt cat ggg cga tga   | 645 |
|    | Ala Lys Gly His Gly Arg *   |     |
|    | 210   |     |
| 35 | <210> 72<br><211> 214<br><212> PRT<br><213> Bordetella pertussis  |     |
| 40 | <400> 72<br>Met Glu Gln Leu Asp Leu Pro Leu Val Val Val Gly Leu Tyr Pro Gly<br>1 5 10 15<br>Met Gln Val Val Leu Ala Ala Val Gly Arg Thr Gly Tyr Asp Pro Gly<br>20 25 30 35<br>Ala Tyr Arg Val Gly Arg Arg Asp Asp His Gly Gly Tyr Arg Arg Ala<br>40 45 50 55 60<br>Gln Phe Asp His Val Leu Ser Ser Pro Gly Thr His Gly Asp Asp Gln<br>65 70 75 80 85<br>Val Asp Val Leu Gly Ala Met Gln Ala Cys Arg Asn Gly Phe Ala His<br>90 95 100 105 110<br>Gly Arg Pro Gln Gly Gly Val Glu Val Ala Gly Leu Pro Pro Arg Ile<br>115 120 125 130 135 140<br>Gly His Glu Leu Asp Val Val Ala Ala Gly Met Arg Gly Gln Val Lys<br>145 150 155 160 165 170 175<br>Pro Gly Leu Ser His Ile Gly Tyr Leu Gly His Val Val Asp Thr Gln<br>180 185 190 195 200 205 210<br>Pro Ser Glu Leu Leu Gln Cys Ala Arg Asp Arg Val Glu Arg Ala Arg<br>215 220 225 230 235 240 245<br>Ile Leu Leu Val Leu Ala Ala Thr His Ile Thr Asp Ala Pro Phe Pro<br>250 255 260 265 270 275 280<br>Ala Gly Cys Met Gly Gly Arg Arg Lys Ala Gly Arg Gly Lys Gly Asp<br>285 290 295 300 305 310 315<br>Ala Gly Ala Gln Gly Arg Asp Arg Arg Ala Gly Cys Arg Arg Asp<br>320 325 330 335 340 345 350<br>Gly Pro Arg Glu Ala Lys Lys Gln Gly Glu Arg Gly Glu Tyr Ser Gly |     |



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|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
|    |     |     |     | 180 |     |     |     | 185 |     |     |     |     | 190 |     |     |     |      |
| 5  | gcc | gtc | tac | acg | cgc | ggc | atg | gtg | cag | cgg | ctg | ctg | atc | ctg | gtc | ggc | 624  |
|    | Ala | Val | Tyr | Thr | Arg | Gly | Met | Val | Gln | Arg | Leu | Leu | Ile | Leu | Val | Gly |      |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |      |
| 10 | ctg | gtg | ctg | gcc | tgc | gtc | atc | tac | gcg | gtc | tgc | gcc | aac | ggc | ctg | ggg | 672  |
|    | Leu | Val | Leu | Ala | Cys | Val | Ile | Tyr | Ala | Val | Cys | Ala | Asn | Gly | Leu | Gly |      |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |      |
| 15 | ctg | ggc | gcg | ccc | atg | gac | ttc | gcc | aag | gtg | gcc | gcc | gcg | ccg | tgg | ttc | 720  |
|    | Leu | Gly | Ala | Pro | Met | Asp | Phe | Ala | Lys | Val | Ala | Ala | Ala | Pro | Trp | Phe |      |
|    |     | 225 |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |      |
| 20 | ggc | ctg | ccc | agc | ttc | gcc | gcg | ccg | gtg | ttc | gag | ccg | cag | gcc | atg | ggc | 768  |
|    | Gly | Leu | Pro | Ser | Phe | Ala | Ala | Pro | Val | Phe | Glu | Pro | Gln | Ala | Met | Gly |      |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |      |
| 25 | ctg | atc | gtg | ccg | gtg | gcc | atc | atc | ctg | gtg | gcc | gag | aac | ctg | ggc | cac | 816  |
|    | Leu | Ile | Val | Pro | Val | Ala | Ile | Ile | Leu | Val | Ala | Glu | Asn | Leu | Gly | His |      |
|    |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |      |
| 30 | gtg | aag | gcg | gtc | gcc | gcc | atg | acc | gga | cag | gac | ctg | gac | cgc | tac | gtg | 864  |
|    | Val | Lys | Ala | Val | Ala | Ala | Met | Thr | Gly | Gln | Asp | Leu | Asp | Arg | Tyr | Val |      |
|    |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |      |
| 35 | ggc | cgc | gcc | ttc | gtg | ggc | gac | ggc | gtg | gcg | acc | atg | gtt | tcc | ggc | gcc | 912  |
|    | Gly | Arg | Ala | Phe | Val | Gly | Asp | Gly | Val | Ala | Thr | Met | Val | Ser | Gly | Ala |      |
|    |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |      |
| 40 | gtc | ggc | ggc | acc | ggg | gtg | acc | acc | tac | gcc | gag | aat | atc | ggc | gtg | atg | 960  |
|    | Val | Gly | Gly | Thr | Gly | Val | Thr | Thr | Tyr | Ala | Glu | Asn | Ile | Gly | Val | Met |      |
|    |     | 305 |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |      |
| 45 | gcc | gtg | acg | cgc | atc | tat | tcc | acg | ctg | gtg | ttc | gtg | gtg | gcg | gcc | gtg | 1008 |
|    | Ala | Val | Thr | Arg | Ile | Tyr | Ser | Thr | Leu | Val | Phe | Val | Val | Ala | Ala | Val |      |
|    |     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |      |
| 50 | atc | gcg | ctg | gtg | ctg | ggg | ttc | tcg | ccc | aag | ttc | ggc | gcg | ctg | atc | cag | 1056 |
|    | Ile | Ala | Leu | Val | Leu | Gly | Phe | Ser | Pro | Lys | Phe | Gly | Ala | Leu | Ile | Gln |      |
|    |     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |      |
| 55 | acc | atc | ccc | ggc | ccc | gtg | ctg | ggg | ggc | atg | tcg | gtc | gtg | gtg | ttc | ggc | 1104 |
|    | Thr | Ile | Pro | Gly | Pro | Val | Leu | Gly | Gly | Met | Ser | Val | Val | Val | Phe | Gly |      |
|    |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |      |
| 60 | ctg | atc | gcc | atc | gcc | ggc | gcg | cgc | atc | tgg | gtg | gtc | aac | cag | gtc | gat | 1152 |
|    | Leu | Ile | Ala | Ile | Ala | Gly | Ala | Arg | Ile | Trp | Val | Val | Asn | Gln | Val | Asp |      |
|    |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |      |
| 65 | ttc | agc | gac | aac | cgc | aat | ctg | atc | gtg | gcc | gcc | gtg | acc | ctg | gtg | ctg | 1200 |
|    | Phe | Ser | Asp | Asn | Arg | Asn | Leu | Ile | Val | Ala | Ala | Val | Thr | Leu | Val | Leu |      |
|    |     | 385 |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |      |
| 70 | ggg | gcg | ggc | gac | ttc | agc | gtc | aag | ctg | ggc | gat | ttc | tcg | atg | aac | ggc | 1248 |
|    | Gly | Ala | Gly | Asp | Phe | Ser | Val | Lys | Leu | Gly | Asp | Phe | Ser | Met | Asn | Gly |      |
|    |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     |     | 415 |     |      |
| 75 | atc | ggc | acc | gcc | acg | ttc | ggc | gcc | atc | atc | ctg | tac | gcc | ctg | ctg | ggc | 1296 |
|    | Ile | Gly | Thr | Ala | Thr | Phe | Gly | Ala | Ile | Ile | Leu | Tyr | Ala | Leu | Leu | Gly |      |

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|    |   |                            |     |     |    |     |      |
|----|---|----------------------------|-----|-----|----|-----|------|
|    |   | 420                        |     | 425 |    | 430 |      |
|    |   | ctg gcg cgt cgc cgc tga    |     |     |    |     | 1314 |
| 5  |   | Leu Ala Arg Arg Arg *      |     |     |    |     |      |
|    |   | 435                        |     |     |    |     |      |
|    |   | <210> 74                   |     |     |    |     |      |
|    |   | <211> 437                  |     |     |    |     |      |
| 10 |   | <212> PRT                  |     |     |    |     |      |
|    |   | <213> Bordetella pertussis |     |     |    |     |      |
|    |   | <400> 74                   |     |     |    |     |      |
| 15 | Met Ser Asn Thr Tyr Phe Pro Arg Trp Arg Leu Ala Asp Asp Thr Val | 1                          | 5   | 10  | 15 |     |      |
|    | Pro Gly Ala Val Ile Ala Pro Asp Glu Arg Leu Ser Trp Pro Lys Asn | 20                         | 25  | 30  |    |     |      |
|    | Ile Ala Met Gly Ala Gln His Val Val Ala Met Phe Gly Ser Thr Val | 35                         | 40  | 45  |    |     |      |
| 20 | Leu Ala Pro Leu Leu Met Gly Phe Asp Pro Asn Val Ala Ile Leu Met | 50                         | 55  | 60  |    |     |      |
|    | Ser Gly Ile Gly Thr Leu Ile Phe Phe Leu Phe Val Gly Gly Arg Val | 65                         | 70  | 75  |    |     |      |
|    | Pro Ser Tyr Leu Gly Ser Ser Phe Ala Phe Ile Gly Gly Val Val Ala | 85                         | 90  | 95  |    |     |      |
| 25 | Val Thr Gly Tyr Val Ala Pro Gly Ala Asn Ala Asn Ile Gly Val Ala | 100                        | 105 | 110 |    |     |      |
|    | Leu Gly Ala Ile Ile Ala Cys Gly Leu Val Tyr Ala Leu Ile Gly Leu | 115                        | 120 | 125 |    |     |      |
| 30 | Val Val Trp Ala Ala Ser Ala Arg Gly Asn Gly Ala Arg Trp Ile Glu | 130                        | 135 | 140 |    |     |      |
|    | Ala Met Met Pro Pro Val Val Thr Gly Ala Val Val Ala Val Ile Gly | 145                        | 150 | 155 |    |     |      |
|    | Leu Asn Leu Ala Pro Ile Ala Ala Lys Gly Ala Met Gly Ser Ser Gly | 165                        | 170 | 175 |    |     |      |
| 35 | Phe Glu Ala Ser Met ala Leu Met Thr Ile Leu Cys Val Gly Gly Ile | 180                        | 185 | 190 |    |     |      |
|    | Ala Val Tyr Thr Arg Gly Met Val Gln Arg Leu Leu Ile Leu Val Gly | 195                        | 200 | 205 |    |     |      |
| 40 | Leu Val Leu Ala Cys Val Ile Tyr Ala Val Cys Ala Asn Gly Leu Gly | 210                        | 215 | 220 |    |     |      |
|    | Leu Gly Ala Pro Met Asp Phe Ala Lys Val Ala Ala Ala Pro Trp Phe | 225                        | 230 | 235 |    |     |      |
|    | Gly Leu Pro Ser Phe Ala Ala Pro Val Phe Glu Pro Gln Ala Met Gly | 245                        | 250 | 255 |    |     |      |
| 45 | Leu Ile Val Pro Val Ala Ile Ile Leu Val Ala Glu Asn Leu Gly His | 260                        | 265 | 270 |    |     |      |
|    | Val Lys Ala Val Ala Ala Met Thr Gly Gln Asp Leu Asp Arg Tyr Val | 275                        | 280 | 285 |    |     |      |
| 50 | Gly Arg Ala Phe Val Gly Asp Gly Val Ala Thr Met Val Ser Gly Ala | 290                        | 295 | 300 |    |     |      |
|    | Val Gly Gly Thr Gly Val Thr Thr Tyr Ala Glu Asn Ile Gly Val Met | 305                        | 310 | 315 |    |     |      |
|    | Ala Val Thr Arg Ile Tyr Ser Thr Leu Val Phe Val Val Ala Ala Val | 325                        | 330 | 335 |    |     |      |
| 55 | Ile Ala Leu Val Leu Gly Phe Ser Pro Lys Phe Gly Ala Leu Ile Gln | 340                        | 345 | 350 |    |     |      |
|    | Thr Ile Pro Gly Pro Val Leu Gly Gly Met Ser Val Val Val Phe Gly | 355                        | 360 | 365 |    |     |      |
| 60 | Leu Ile Ala Ile Ala Gly Ala Arg Ile Trp Val Val Asn Gln Val Asp |                            |     |     |    |     |      |

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|    |   |                 |                         |     |     |     |
|----|---|-----------------|-------------------------|-----|-----|-----|
|    | 370   |                 | 375                     |     | 380 |     |
|    | Phe Ser Asp Asn Arg Asn   | Leu Ile Val Ala | Ala Val Thr Leu Val Leu |     |     |     |
|    | 385   | 390             | 395                     | 400 |     |     |
| 5  | Gly Ala Gly Asp Phe Ser Val Lys Leu Gly Asp Phe Ser Met Asn Gly |                 |                         |     |     |     |
|    | 405   | 410             | 415                     |     |     |     |
|    | Ile Gly Thr Ala Thr Phe Gly Ala Ile Ile Leu Tyr Ala Leu Leu Gly |                 |                         |     |     |     |
|    | 420   | 425             | 430                     |     |     |     |
|    | Leu Ala Arg Arg Arg   |                 |                         |     |     |     |
|    | 435   |                 |                         |     |     |     |
| 10 |   |                 |                         |     |     |     |
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|    | <212> DNA   |                 |                         |     |     |     |
| 15 | <213> Bordetella pertussis                                      |                 |                         |     |     |     |
|    | <220>   |                 |                         |     |     |     |
|    | <221> CDS   |                 |                         |     |     |     |
|    | <222> (1)...(1536)  |                 |                         |     |     |     |
| 20 |   |                 |                         |     |     |     |
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|    | atg ctt gaa agg atc aag gtc cgc acc gcg atg gtg gcg gta ttc gcg |                 |                         |     |     | 48  |
|    | Met Leu Glu Arg Ile Lys Val Arg Thr Ala Met Val Ala Val Phe Ala |                 |                         |     |     |     |
|    | 1 5 10 15   |                 |                         |     |     |     |
| 25 |   |                 |                         |     |     |     |
|    | tgc ttc ctg gcg gtg ctg atg ctg tcg ggc gcc ctg acg tgg cgc aac |                 |                         |     |     | 96  |
|    | Cys Phe Leu Ala Val Leu Met Leu Ser Gly Ala Leu Thr Trp Arg Asn |                 |                         |     |     |     |
|    | 20 25 30  |                 |                         |     |     |     |
| 30 |   |                 |                         |     |     |     |
|    | gcg ggc agg agc gcc gcc gag atc gag ggg ctg aac cag gtc gcc gtc |                 |                         |     |     | 144 |
|    | Ala Gly Arg Ser Ala Ala Glu Ile Glu Gly Leu Asn Gln Val Ala Val |                 |                         |     |     |     |
|    | 35 40 45  |                 |                         |     |     |     |
| 35 |   |                 |                         |     |     |     |
|    | aac cag gtc gac ccg ctg ttc gag gcc agc ggc gcg gcg cag cgc cag |                 |                         |     |     | 192 |
|    | Asn Gln Val Asp Pro Leu Phe Glu Ala Ser Gly Ala Ala Gln Arg Gln |                 |                         |     |     |     |
|    | 50 55 60  |                 |                         |     |     |     |
| 40 |   |                 |                         |     |     |     |
|    | gcg gcc acg caa ttc cag cgc tac gtg gac gtg ccc aag gag ccg gcc |                 |                         |     |     | 240 |
|    | Ala Ala Thr Gln Phe Gln Arg Tyr Val Asp Val Pro Lys Glu Pro Ala |                 |                         |     |     |     |
|    | 65 70 75 80   |                 |                         |     |     |     |
| 45 |   |                 |                         |     |     |     |
|    | gcg gcc gag ctg gcc gcg acc ctg cag acg cgc tgg cgc gcc tac cag |                 |                         |     |     | 288 |
|    | Ala Ala Glu Leu Ala Thr Leu Gln Thr Arg Trp Arg Ala Tyr Gln     |                 |                         |     |     |     |
|    | 85 90 95  |                 |                         |     |     |     |
| 50 |   |                 |                         |     |     |     |
|    | tcg gtg ctg gac gag ctg gcc gcc gcc gtc gac gcc ggc cag gcc gag |                 |                         |     |     | 336 |
|    | Ser Val Leu Asp Glu Leu Ala Ala Val Asp Ala Gly Gln Ala Glu     |                 |                         |     |     |     |
|    | 100 105 110   |                 |                         |     |     |     |
| 55 |   |                 |                         |     |     |     |
|    | ccc gcc ctg gcc gcc atg cat cgc gcg cag cag gcc gaa cat gca ttc |                 |                         |     |     | 384 |
|    | Pro Ala Leu Ala Ala Met His Arg Ala Gln Gln Ala Glu His Ala Phe |                 |                         |     |     |     |
|    | 115 120 125   |                 |                         |     |     |     |
| 60 |   |                 |                         |     |     |     |
|    | cag cgc gac atg gaa gcc ttt ctg gcc agg gta cag gcg cac agc gac |                 |                         |     |     | 432 |
|    | Gln Arg Asp Met Glu Ala Phe Leu Ala Arg Val Gln Ala His Ser Asp |                 |                         |     |     |     |
|    | 130 135 140   |                 |                         |     |     |     |
| 65 |   |                 |                         |     |     |     |
|    | gaa gtg cgc agc gcc gcc gag gac acc cat gtc gtg gcc cgc tgg agc |                 |                         |     |     | 480 |
|    | Glu Val Arg Ser Gly Ala Glu Asp Thr His Val Val Ala Arg Trp Ser |                 |                         |     |     |     |
|    | 145 150 155 160   |                 |                         |     |     |     |

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|    |   |      |
|----|---|------|
|    | gcc atc gcg ctg acc acg ctg ggc gtg ctg ctg acc ctg gcc ggc tgg | 528  |
|    | Ala Ile Ala Leu Thr Thr Leu Gly Val Leu Leu Thr Leu Ala Gly Trp |      |
|    | 165 170 175   |      |
| 5  | ctg ttc gtg cgc cgc gcg gtg ctg cgc ccc ttg ctg gag gcc ggc cat | 576  |
|    | Leu Phe Val Arg Arg Ala Val Leu Arg Pro Leu Leu Glu Ala Gly His |      |
|    | 180 185 190   |      |
| 10 | cat ttc gac cgc atc gcc gac ggc gac ctc acc gcg cgc atc gag gtg | 624  |
|    | His Phe Asp Arg Ile Ala Asp Gly Asp Leu Thr Ala Arg Ile Glu Val |      |
|    | 195 200 205   |      |
| 15 | cgc tcg gcc aat gaa atc ggc gcg ctg ttc gcg gcg ctc aag cgc atg | 672  |
|    | Arg Ser Ala Asn Glu Ile Gly Ala Leu Phe Ala Ala Leu Lys Arg Met |      |
|    | 210 215 220   |      |
| 20 | cag gaa ggc ctg acg cgc acc atc gcc gtc atg cgg cgc ggc gtc gac | 720  |
|    | Gln Glu Gly Leu Thr Arg Thr Ile Ala Val Met Arg Arg Gly Val Asp |      |
|    | 225 230 235 240   |      |
| 25 | gaa atc aac gtc ggc gcg gcc gag atc tcg gcc ggc aac gcc aac ctg | 768  |
|    | Glu Ile Asn Val Gly Ala Ala Glu Ile Ser Ala Gly Asn Ala Asn Leu |      |
|    | 245 250 255   |      |
| 30 | tcc agc cgc acg gag gag cag gcc gcc gcc ctg gaa gag acc gcg gcc | 816  |
|    | Ser Ser Arg Thr Glu Glu Gln Ala Ala Ala Leu Glu Glu Thr Ala Ala |      |
|    | 260 265 270   |      |
| 35 | acc atg gag gaa ctg gcc acc acg gtc aag cag aac gcc gac aat gcc | 864  |
|    | Thr Met Glu Glu Leu Ala Thr Thr Val Lys Gln Asn Ala Asp Asn Ala |      |
|    | 275 280 285   |      |
| 40 | gcg cag gcc aat cag ctg gcc gcc gtc agc atg cag gtg gcg cag cgc | 912  |
|    | Ala Gln Ala Asn Gln Leu Ala Ala Val Ser Met Gln Val Ala Gln Arg |      |
|    | 290 295 300   |      |
| 45 | ggc ggc gag tcg gtc gcg cag gtg gtg cag acc atg cac ggc atc tcc | 960  |
|    | Gly Gly Glu Ser Val Ala Gln Val Val Gln Thr Met His Gly Ile Ser |      |
|    | 305 310 315 320   |      |
| 50 | gcg agc tcg cgc cag atc gcc gac atc gtc acc gtg atc gac ggc atc | 1008 |
|    | Ala Ser Ser Arg Gln Ile Ala Asp Ile Val Thr Val Ile Asp Gly Ile |      |
|    | 325 330 335   |      |
| 55 | gcc ttc cag acc aat atc ctg gcg ctg aac gcc gcg gtc gag gcg gcg | 1056 |
|    | Ala Phe Gln Thr Asn Ile Leu Ala Leu Asn Ala Ala Val Glu Ala Ala |      |
|    | 340 345 350   |      |
| 60 | cgc gcc ggc gaa cag ggc aag ggc ttc gcg gtg gtg gcg ggc gag gtg | 1104 |
|    | Arg Ala Gly Glu Gln Gly Lys Gly Phe Ala Val Val Ala Gly Glu Val |      |
|    | 355 360 365   |      |
| 65 | cgc agc ctg gcc cag cgc gcc gcg cag gcg gcc aag gag atc aag gcc | 1152 |
|    | Arg Ser Leu Ala Gln Arg Ala Ala Gln Ala Ala Lys Glu Ile Lys Ala |      |
|    | 370 375 380   |      |
| 70 | ctg atc gag agc tcg gtg gcg acg gtg cgc gcc ggc tcg caa cag gtc | 1200 |
|    | Leu Ile Glu Ser Ser Val Ala Thr Val Arg Ala Gly Ser Gln Gln Val |      |
|    | 385 390 395 400   |      |



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|    |   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|----|---|------|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|
|    | gcc agc gcc ggc ggc acc atg gac gag gtg gtg gcc tcg gta cag cgc | 1248 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Ala Ser Ala Gly Gly Thr Met Asp Glu Val Val Ala Ser Val Gln Arg |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 405 410 415   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 5  | gtg gcc gac atc atg ggg gag atc tcg gcc gcc tcg gcc cag cag gcc | 1296 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Val Ala Asp Ile Met Gly Glu Ile Ser Ala Ala Ser Ala Gln Gln Ala |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 420 425 430   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 10 | agc ggc atc gac cag gtc agc ctg gcg att tcg caa atg gac gaa acc | 1344 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Ser Gly Ile Asp Gln Val Ser Leu Ala Ile Ser Gln Met Asp Glu Thr |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 435 440 445   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 15 | acc cag cag aat gcc gcg ctg gtc gaa cag gcc gcg gcg gcg gcc acg | 1392 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Thr Gln Gln Asn Ala Ala Leu Val Glu Gln Ala Ala Ala Ala Thr     |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 450 455 460   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 20 | gcc atg gaa gaa cag gcc cgc cac ctg gcg gcc gcg gcg gcg gtc ttc | 1440 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Ala Met Glu Glu Gln Ala Arg His Leu Ala Ala Ala Ala Val Phe     |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 465 470 475 480   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | agg acg cag ggc ggc gcc atc atc gac gtc gcc gcc gcg ccg ctg gcc | 1488 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Arg Thr Gln Gly Gly Ala Ile Ile Asp Val Ala Ala Ala Pro Leu Ala |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 485 490 495   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 25 | ggg ccg gcg ggc ggc cat gcc gcc ctg ccg ccg gcc gcg gcc cac tga | 1536 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Gly Pro Ala Gly Gly His Ala Ala Leu Pro Pro Ala Ala Ala His *   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 500 505 510   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 30 | <210> 76  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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|    | <212> PRT   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | <213> Bordetella pertussis                                      |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 35 | <400> 76  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Met Leu Glu Arg Ile Lys Val Arg Thr Ala Met Val Ala Val Phe Ala |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 1 5 10 15   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Cys Phe Leu Ala Val Leu Met Leu Ser Gly Ala Leu Thr Trp Arg Asn |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 20 25 30  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 40 | Ala Gly Arg Ser Ala Ala Glu Ile Glu Gly Leu Asn Gln Val Ala Val |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 35 40 45  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Asn Gln Val Asp Pro Leu Phe Glu Ala Ser Gly Ala Ala Gln Arg Gln |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 50 55 60  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 45 | Ala Ala Thr Gln Phe Gln Arg Tyr Val Asp Val Pro Lys Glu Pro Ala |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 65 70 75 80   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Ala Ala Glu Leu Ala Ala Thr Leu Gln Thr Arg Trp Arg Ala Tyr Gln |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 85 90 95  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Ser Val Leu Asp Glu Leu Ala Ala Ala Val Asp Ala Gly Gln Ala Glu |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 100 105 110   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 50 | Pro Ala Leu Ala Ala Met His Arg Ala Gln Gln Ala Glu His Ala Phe |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 115 120 125   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Gln Arg Asp Met Glu Ala Phe Leu Ala Arg Val Gln Ala His Ser Asp |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 130 135 140   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 55 | Glu Val Arg Ser Gly Ala Glu Asp Thr His Val Val Ala Arg Trp Ser |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 145 150 155 160   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Ala Ile Ala Leu Thr Thr Leu Gly Val Leu Leu Thr Leu Ala Gly Trp |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 165 170 175   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | Leu Phe Val Arg Ala Val Leu Arg Pro Leu Leu Glu Ala Gly His     |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|    | 180 185 190   |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 60 | His Phe Asp Arg Ile Ala Asp Gly Asp Leu Thr Ala Arg Ile Glu Val |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |



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[illegible]

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```

<212> DNA
<213> Bordetella pertussis

<220>
5 <221> CDS
  <222> (1)...(951)

  <400> 79
10 atg aac acc cat aag cat gcc cga ttg acc ttc cta cgt cga ctc gaa      48
   Met Asn Thr His Lys His Ala Arg Leu Thr Phe Leu Arg Arg Leu Glu
    1          5          10          15

15 atg gtc cag caa ttg atc gcc cat caa gtt tgt gtg cct gaa gcg gcc      96
   Met Val Gln Gln Leu Ile Ala His Gln Val Cys Val Pro Glu Ala Ala
          20          25          30

20 cgc gcc tat ggg gtc acc gcg ccg act gtg cgc aaa tgg ctg ggc cgc      144
   Arg Ala Tyr Gly Val Thr Ala Pro Thr Val Arg Lys Trp Leu Gly Arg
          35          40          45

25 ttc ctg gct cag ggc cag gcg ggc ttg gcc gat gcg tcc tcg cgc ccg      192
   Phe Leu Ala Gln Gly Gln Ala Gly Leu Ala Asp Ala Ser Ser Arg Pro
          50          55          60

30 acg gtc tcg ccc cga gcg att gcg ccg gcc aag gcg ctg gct atc gtg      240
   Thr Val Ser Pro Arg Ala Ile Ala Pro Ala Lys Ala Leu Ala Ile Val
    65          70          75          80

35 gag ctg cgc cgc aag cgg ctg acc caa gcg cgc atc gcc cag gcg ctg      288
   Glu Leu Arg Arg Lys Arg Leu Thr Gln Ala Arg Ile Ala Gln Ala Leu
          85          90          95

40 ggc gtg tca gcc agc acc gtc agc cgc gtc ctg gcc cgc gcc ggt ctg      336
   Gly Val Ser Ala Ser Thr Val Ser Arg Val Leu Ala Arg Ala Gly Leu
          100          105          110

45 teg cac ctg gcc gac ctg gag ccg gcc gag ccg gtg gtg cgc tac gag      384
   Ser His Leu Ala Asp Leu Glu Pro Ala Glu Pro Val Val Arg Tyr Glu
          115          120          125

50 cat cag gcc ccc ggc gat ctg ctg cac atc gac atc aag aag ctg gga      432
   His Gln Ala Pro Gly Asp Leu Leu His Ile Asp Ile Lys Lys Leu Gly
          130          135          140

55 cgt atc cag cgc cct ggc cac cgg gtc acg ggc aac cga cgc gat acc      480
   Arg Ile Gln Arg Pro Gly His Arg Val Thr Gly Asn Arg Arg Asp Thr
          145          150          155          160

60 gtt gag ggg gcc ggc tgg gac ttc gtc ttc gtg gcc atc gat gac cac      528
   Val Glu Gly Ala Gly Trp Asp Phe Val Phe Val Ala Ile Asp Asp His
          165          170          175

   gcc cgc gtg gcc ttc acc gac atc cac ccc gac gag cgc ttc ccc agc      576
   Ala Arg Val Ala Phe Thr Asp Ile His Pro Asp Glu Arg Phe Pro Ser
          180          185          190

   gcc gtc cag ttc ctc aag gac gca gtg gcc tac tac cag cgc ctg ggc      624
   Ala Val Gln Phe Leu Lys Asp Ala Val Ala Tyr Tyr Gln Arg Leu Gly
          195          200          205

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|    |   |     |
|----|---|-----|
|    | gtg acc atc cag cgc ttg ctc acc gac aat ggc tcg gcc ttt cgc agc | 672 |
|    | Val Thr Ile Gln Arg Leu Leu Thr Asp Asn Gly Ser Ala Phe Arg Ser |     |
|    | 210 215 220   |     |
| 5  | cgc gcc ttc gcc gcg ctg tgc cat gag ctg ggc atc aag cac cgc ttt | 720 |
|    | Arg Ala Phe Ala Ala Leu Cys His Glu Leu Gly Ile Lys His Arg Phe |     |
|    | 225 230 235 240   |     |
| 10 | acc cga cct tac cgc cca cag acc aat ggc aag gcc gaa cgc ttc atc | 768 |
|    | Thr Arg Pro Tyr Arg Pro Gln Thr Asn Gly Lys Ala Glu Arg Phe Ile |     |
|    | 245 250 255   |     |
| 15 | cag tcg gcc ttg cgt gag tgg gct tac gct cac acc tac cag aac tcc | 816 |
|    | Gln Ser Ala Leu Arg Glu Trp Ala Tyr Ala His Thr Tyr Gln Asn Ser |     |
|    | 260 265 270   |     |
| 20 | caa cac cga gcc gat gcc atg aaa tcc tgg cta cac cac tac aac tgg | 864 |
|    | Gln His Arg Ala Asp Ala Met Lys Ser Trp Leu His His Tyr Asn Trp |     |
|    | 275 280 285   |     |
| 25 | cat cga ccc cac caa ggc atc ggg cgc gct gta ccc atc tcc aga ctc | 912 |
|    | His Arg Pro His Gln Gly Ile Gly Arg Ala Val Pro Ile Ser Arg Leu |     |
|    | 290 295 300   |     |
| 30 | aac ctg gac gaa tac aac cta ttg aca gtt cac acc tag             | 951 |
|    | Asn Leu Asp Glu Tyr Asn Leu Leu Thr Val His Thr *               |     |
|    | 305 310 315   |     |
| 30 | <210> 80  |     |
|    | <211> 316   |     |
|    | <212> PRT   |     |
|    | <213> Bordetella pertussis                                      |     |
| 35 | <400> 80  |     |
|    | Met Asn Thr His Lys His Ala Arg Leu Thr Phe Leu Arg Arg Leu Glu |     |
|    | 1 5 10 15   |     |
|    | Met Val Gln Gln Leu Ile Ala His Gln Val Cys Val Pro Glu Ala Ala |     |
|    | 20 25 30  |     |
| 40 | Arg Ala Tyr Gly Val Thr Ala Pro Thr Val Arg Lys Trp Leu Gly Arg |     |
|    | 35 40 45  |     |
|    | Phe Leu Ala Gln Gly Gln Ala Gly Leu Ala Asp Ala Ser Ser Arg Pro |     |
|    | 50 55 60  |     |
| 45 | Thr Val Ser Pro Arg Ala Ile Ala Pro Ala Lys Ala Leu Ala Ile Val |     |
|    | 65 70 75 80   |     |
|    | Glu Leu Arg Arg Lys Arg Leu Thr Gln Ala Arg Ile Ala Gln Ala Leu |     |
|    | 85 90 95  |     |
|    | Gly Val Ser Ala Ser Thr Val Ser Arg Val Leu Ala Arg Ala Gly Leu |     |
|    | 100 105 110   |     |
| 50 | Ser His Leu Ala Asp Leu Glu Pro Ala Glu Pro Val Val Arg Tyr Glu |     |
|    | 115 120 125   |     |
|    | His Gln Ala Pro Gly Asp Leu Leu His Ile Asp Ile Lys Lys Leu Gly |     |
|    | 130 135 140   |     |
| 55 | Arg Ile Gln Arg Pro Gly His Arg Val Thr Gly Asn Arg Arg Asp Thr |     |
|    | 145 150 155 160   |     |
|    | Val Glu Gly Ala Gly Trp Asp Phe Val Phe Val Ala Ile Asp Asp His |     |
|    | 165 170 175   |     |
|    | Ala Arg Val Ala Phe Thr Asp Ile His Pro Asp Glu Arg Phe Pro Ser |     |
|    | 180 185 190   |     |
| 60 | Ala Val Gln Phe Leu Lys Asp Ala Val Ala Tyr Tyr Gln Arg Leu Gly |     |





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|    | 370   | 375 | 380 |      |
|----|---|-----|-----|------|
| 5  | atc caa ctt ggc ctg ctt ccc cag ccg ttg ccc gat gcg gcc acg cgc<br>Ile Gln Leu Gly Leu Leu Pro Gln Pro Leu Pro Asp Ala Ala Thr Arg<br>385 390 395 400 |     |     | 1200 |
| 10 | ggc agc cag ttg cgt gcc gtc atg tac ccg gcc cgg gag gtc ggt ggg<br>Gly Ser Gln Leu Arg Ala Val Met Tyr Pro Ala Arg Glu Val Gly Gly<br>405 410 415     |     |     | 1248 |
|    | gat ttc tac gac tac ttc gtg ctg gca gac ggg cgt ctg tgc ttt gcc<br>Asp Phe Tyr Asp Tyr Phe Val Leu Ala Asp Gly Arg Leu Cys Phe Ala<br>420 425 430     |     |     | 1296 |
| 15 | atc ggc gac gta tcc gga aaa ggc gtg ccc gcg gcc ctg ttc atg gcc<br>Ile Gly Asp Val Ser Gly Lys Gly Val Pro Ala Ala Leu Phe Met ala<br>435 440 445     |     |     | 1344 |
| 20 | atc gtc agg acc ttg ata cgc agc gtg gcg gaa gaa gag cac gac ccg<br>Ile Val Arg Thr Leu Ile Arg Ser Val Ala Glu Glu Glu His Asp Pro<br>450 455 460     |     |     | 1392 |
| 25 | ggc gcc atc gcc acc aag gtg aac cac cgt ctg gcc gag aac aac ccc<br>Gly Ala Ile Ala Thr Lys Val Asn His Arg Leu Ala Glu Asn Asn Pro<br>465 470 475 480 |     |     | 1440 |
| 30 | aag ctg atg ttt gtc acc ttg ctg ata ggc gtc ttc acc ccg gaa aca<br>Lys Leu Met Phe Val Thr Leu Leu Ile Gly Val Phe Thr Pro Glu Thr<br>485 490 495     |     |     | 1488 |
|    | ggc gcc ctg gcc tgg gtc aac gcc ggc cac ccg ccg ccg ctg ctc atc<br>Gly Ala Leu Ala Trp Val Asn Ala Gly His Pro Pro Pro Leu Leu Ile<br>500 505 510     |     |     | 1536 |
| 35 | gac gaa cgt ggc gag gtc cgc ctg ctt caa gga agc agc ggc gcg gcc<br>Asp Glu Arg Gly Glu Val Arg Leu Leu Gln Gly Ser Ser Gly Ala Ala<br>515 520 525     |     |     | 1584 |
| 40 | tgc ggc gtg ctg gac aac gag gcg tat tcc acc ctg agc acc acc ttg<br>Cys Gly Val Leu Asp Asn Glu Ala Tyr Ser Thr Leu Ser Thr Thr Leu<br>530 535 540     |     |     | 1632 |
| 45 | ccg aac ggc acc tcg ctg gtc gcg ttt acc gac ggc gtc acc gaa gcc<br>Pro Asn Gly Thr Ser Leu Val Ala Phe Thr Asp Gly Val Thr Glu Ala<br>545 550 555 560 |     |     | 1680 |
| 50 | atc cac ggc ggc tgc gcc cag tat ggt ctg ccg ccg ctg gtc gcc ctg<br>Ile His Gly Gly Cys Ala Gln Tyr Gly Leu Pro Arg Leu Val Ala Leu<br>565 570 575     |     |     | 1728 |
|    | atg cag ggc gcg ccg cac gca gcg gcc gaa ctc atc gag cac att ctg<br>Met Gln Gly Ala Pro His Ala Ala Ala Glu Leu Ile Glu His Ile Leu<br>580 585 590     |     |     | 1776 |
| 55 | cac gac cta cgc gaa ttc gcc gcc gat tcc gaa caa tcc gac gat ctc<br>His Asp Leu Arg Glu Phe Ala Ala Asp Ser Glu Gln Ser Asp Asp Leu<br>595 600 605     |     |     | 1824 |
| 60 | acc atc atc gcc att cat cgc cca tga<br>Thr Ile Ile Ala Ile His Arg Pro *  |     |     | 1851 |



610 615

5 <210> 82  
 <211> 616  
 <212> PRT  
 <213> Bordetella pertussis

10 <400> 82  
 Met Asp Leu Val Val Arg Asp Thr Asp Thr Arg Trp Ser Thr Leu Leu  
 1 5 10  
 Asp Asp Lys Ile Arg Thr Ile Arg Glu Ser Arg Arg Gln Leu Ile Gln  
 20 25 30  
 Leu Ser Ala Val Val Thr Ser Val Leu Asn Ala Tyr Ala Gln Ala  
 35 40 45  
 15 Glu Arg Gly His Val Thr Thr Gly Ala Ala Lys Gly Met ala Arg Val  
 50 55 60  
 Trp Leu Asn His Leu Asp Leu Gly Pro Arg Arg Val Ala Phe Ala Tyr  
 65 70 75 80  
 20 Asp Ala Glu Gly Thr Val Leu Ala Ser Thr Asn Pro Arg Met Ile Asp  
 85 90 95  
 Arg Asp Leu Ser Gly Ile Arg Asp Phe Lys Gly Arg Pro Leu Ala Ala  
 100 105 110  
 Ala Met Tyr Glu Glu Ser Arg Asn Asp Gly Arg Gly Phe Ala Ile Tyr  
 115 120 125  
 25 Pro Ser Pro Leu Asp Glu Ser Ala Gln Met Arg His Ala Tyr Phe Val  
 130 135 140  
 Tyr Phe Pro Ala Trp Lys Trp Val Leu Ala Ile Ser Asp Ser Ser Gln  
 145 150 155 160  
 30 Ala Ile Ile Asp Lys Val Ala Ala Gln Lys Ala Asn Met Ile Ala Ala  
 165 170 175  
 Ile Asp Arg Asn Leu Ser Glu Leu Arg Leu Ser Arg His Gly Phe Val  
 180 185 190  
 35 Phe Val Val Ala Asp Asp Gly Thr Val Ile Val Pro Pro Pro Ser  
 195 200 205  
 Ala Ala Arg Leu Leu Asp Ser Thr Asp Val Glu Ser Gly Arg Val Leu  
 210 215 220  
 His Ser Met Leu Ala Glu Ile Ser Ser Thr Arg Gly Leu Thr Leu Arg  
 225 230 235 240  
 40 Phe Thr Asn Gly Glu Ser Ala Trp Gln Ile Asp Ala Leu Arg Tyr Lys  
 245 250 255  
 Pro Leu His Trp Thr Ile Ile Gly Val Val Pro Glu Pro Asp Leu Thr  
 260 265 270  
 Asp Pro Ala Gln Asn Leu Val Arg Arg Gln Ala Leu Ile Phe Ala Ala  
 275 280 285  
 45 Thr Leu Leu Ala Gly Leu Met Leu Ala Trp Val Val Ala Val Arg Ile  
 290 295 300  
 Ala Arg Pro Leu Ala Gln Leu Ser Asn Tyr Ala Arg Gln Leu Pro Thr  
 305 310 315 320  
 50 Gln Asp Leu Thr Glu Pro Ile Arg Val Pro Pro Ser Val Ala Cys Leu  
 325 330 335  
 Pro Arg Arg Arg Arg Asp Glu Val Gly Gln Leu Ala Glu Ser Phe Leu  
 340 345 350  
 55 Phe Met Asn Glu Gln Leu His His Asn Val Arg Ala Leu Met ala Gln  
 355 360 365  
 Ile Ser Asn Arg Glu Arg Leu Glu Ser Glu Leu Ser Ile Ala Arg Ser  
 370 375 380  
 Ile Gln Leu Gly Leu Leu Pro Gln Pro Leu Pro Asp Ala Ala Thr Arg  
 385 390 395 400  
 60 Gly Ser Gln Leu Arg Ala Val Met Tyr Pro Ala Arg Glu Val Gly Gly

84

**Amended Claims for PCT Application No. PCT/EP99/10297****Claims:**

1. An isolated polypeptide comprising an amino acid sequence which has at least 75% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72 over its entire length.
2. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72.
3. An isolated polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.
4. An isolated polypeptide comprising a fragment of at least 7 consecutive amino acids of the polypeptide as claimed in any one of claims 1 to 3, wherein the fragment comprises an epitope.
5. The polypeptide of claim 4, wherein the fragment is immunogenic.
6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 75% identity to the amino acid sequence of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.
7. An isolated polynucleotide comprising a nucleotide sequence that has at least 75% identity to a nucleotide sequence, encoding a polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

8. An isolated polynucleotide which comprises a nucleotide sequence which has at least 75% identity to that of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

9. The isolated polynucleotide as claimed in any one of claims 6 to 8 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

11. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71.

12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 or a fragment thereof.

13. An expression vector comprising an isolated polynucleotide according to any one of claims 6 - 12.

14. A recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 6 - 12.

15. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell.

- AMENDED SHEET

24. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 - 5 in the preparation of a medicament for use in generating an immune response in an animal.

25. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 6 - 12 in the preparation of a medicament for use in generating an immune response in an animal.

26. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claims 1 - 5 and a suitable pharmaceutical carrier.

27. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polynucleotide of claims 6-12 or a polypeptide of claims 1-5.

28. A method of identifying virulence genes from a pathogenicity island containing a type III secretion system from pathogenic strains of bacteria, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers of *lcrD*-like genes present in said pathogenic strain of bacteria;

sequencing the *lcrD*-like gene;

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes; and

if a virulence-associated member, sequencing the entire pathogenicity island, and

identifying genes within this sequence.

29. A method of determining whether a particular bacterial strain harbours a type III secretion system involved in pathogenicity, comprising:

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designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;  
amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers to determine the presence of any *lcrD*-like genes in said bacterial strain;  
if amplified successfully, sequencing the *lcrD*-like gene; and  
determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <p>(51) International Patent Classification <sup>7</sup> :<br/>C07K 14/235, C12N 15/31, 5/10, A61K 39/10, C07K 16/12, G01N 33/569, C12Q 1/68</p>  | <p>A2</p> | <p>(11) International Publication Number: <b>WO 00/37493</b><br/>(43) International Publication Date: 29 June 2000 (29.06.00)</p>   |
| <p>(21) International Application Number: PCT/EP99/10297<br/>(22) International Filing Date: 21 December 1999 (21.12.99)<br/>(30) Priority Data:<br/>9828217.1 21 December 1998 (21.12.98) GB<br/>(71) Applicant (for all designated States except US): UNIVERSITE LIBRE DE BRUXELLES [BE/BE]; Faculté des Sciences, Avenue F. Roosevelt, 50, B-1050 Bruxelles (BE).<br/>(72) Inventors; and<br/>(75) Inventors/Applicants (for US only): BOLLEN, Alex [BE/BE]; Université Libre de Bruxelles, Faculté des Sciences, Avenue F. Roosevelt, 50, B-1050 Bruxelles (BE). FAUCONNIER, Alain [BE/BE]; Université Libre de Bruxelles, Faculté des Sciences, Avenue F. Roosevelt, 50, B-1050 Bruxelles (BE). GODFROID, Edmond [BE/BE]; Université Libre de Bruxelles, Faculté des Sciences, Avenue F. Roosevelt, 50, B-1050 Bruxelles (BE).<br/>(74) Agent: TYRRELL, William, Arthur, Russell; SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).</p> |           | <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b><br/><i>Without international search report and to be republished upon receipt of that report.</i></p> |
| <p>(54) Title: VACCINE</p> <p>(57) Abstract</p> <p>This invention relates to a general method for detecting pathogenic strains of bacteria which harbour a type III secretion system. More particularly, this invention relates to the methods as applied to the pathogen <i>Bordetella pertussis</i>. Furthermore, the invention relates to newly identified polynucleotides within these regions, virulent polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly the polynucleotides and polypeptides of the present invention relate to the virulent effector proteins associated with the type III secretion system of <i>Bordetella pertussis</i>, which are particularly suitable for vaccine purposes.</p>  |           |   |



**Fig. 1**

```

GGAATGCCAGGAAAGCAGATGTCCATCGACGCGGACTTGCGCGCGGGCACCATAGACATG
1  -----+-----+-----+-----+-----+-----+ 60
CCTTACGGTCTTTTCGTCTACAGGTAGCTGCGCCTGAACGCGCGCCCGTGGTATCTGTAC

G M P G K Q M S I D A D L R A G T I D M -

GACGAAGCCCGACGCCGACGCGGTACGGTCGAGAAGGAAAGCCAACTGTATGGCGCCATG
61 -----+-----+-----+-----+-----+-----+ 120
CTGCTTCGGGCTGCGGCTGCGGCATGCCAGCTCTTCCTTTCGGTTGACATACCGCGGTAC

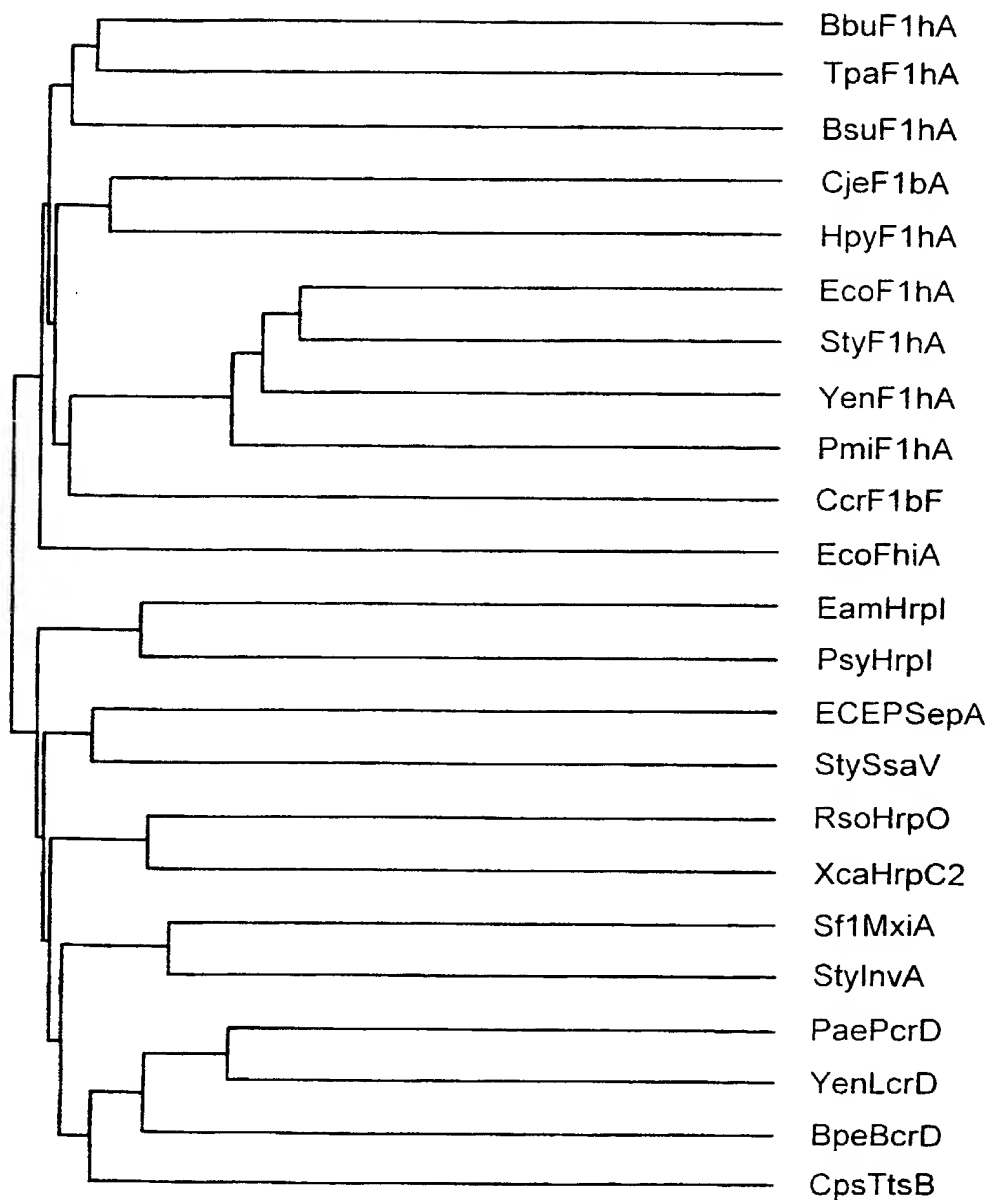
D E A R R R R R T V E K E S Q L Y G A M -

GACGGCGCGATGAAATTTGTCAAGGGCGACGC
121 -----+-----+-----+-----+ 152
CTGCCGCGCTACTTTAAACAGTTCCCGCTGCG

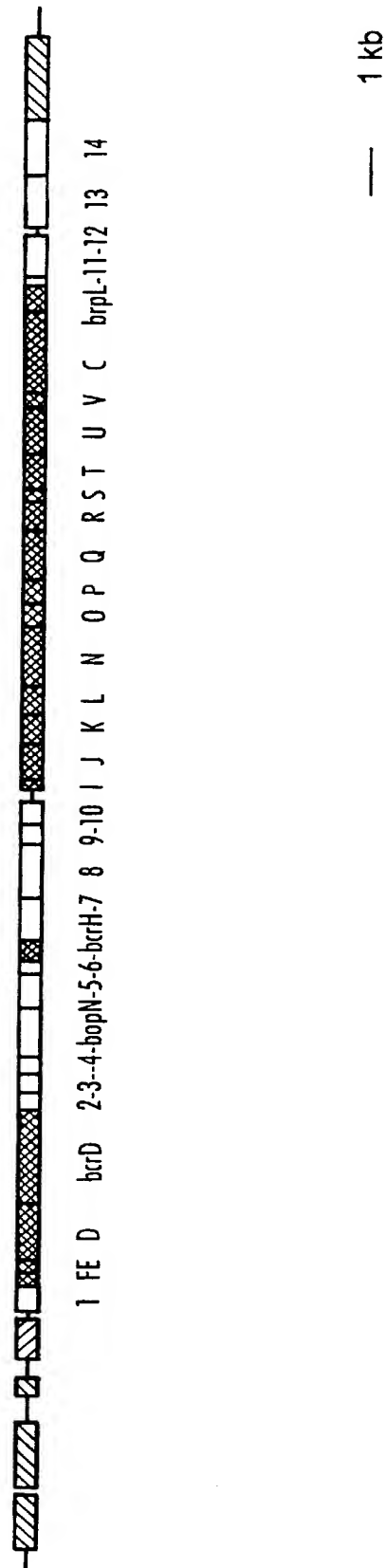
D G A M K F V K G D A -

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**Fig. 2**



**Fig. 3**



**Fig. 4**

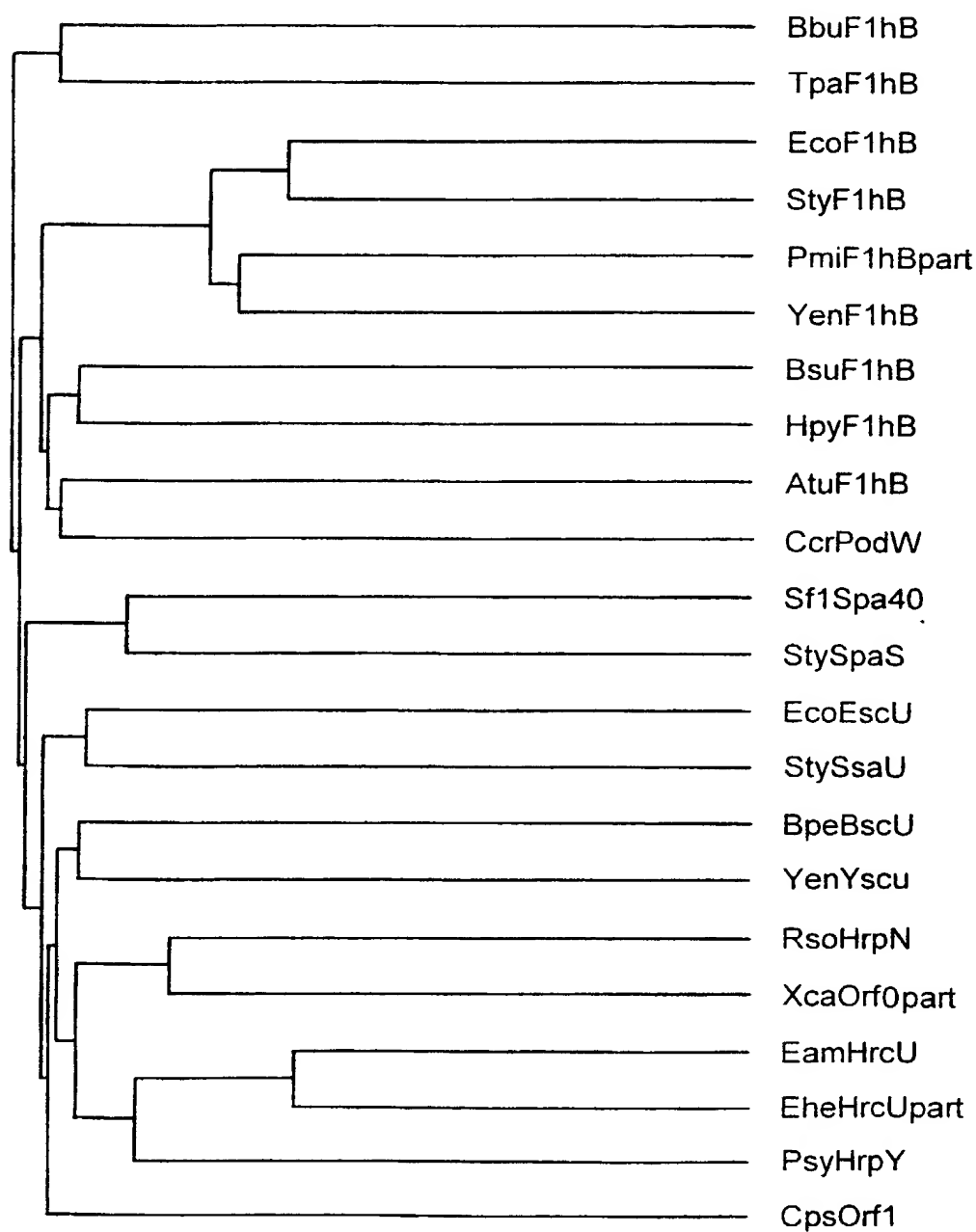




Figure 5 (continued)

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 1551 GGACAGGACC TGGACCGCTA CGTGGGCGCG GCCTTCGTGG GCGACGGCGT  
 1601 GCGGACCATG GTTTCGGCG CCGTCGGCGG CACCGGGGTG ACCACCTACG  
 1651 CCGAGAATAT CGGCGTGATG GCCGTGACGC GCATCTATTC CACGCTGGTG  
 1701 TTCGTGGTGG CGGCCGTGAT CGCGCTGGTG CTGGGGTTCT CGCCCAAGTT  
 1751 CGGCGCGCTG ATCCAGACCA TCCCCGGCCC CGTGCTGGGG GGCATGTCGG  
 1801 TCGTGGTGTT CGGCCTGATC GCCATCGCCG GCGCGCGCAT CTGGGTGGTC  
 1851 AACCAGGTCG ATTTTCAGCGA CAACCGCAAT CTGATCGTGG CCGCCGTGAC  
 1901 CCTGGTGCTG GGGGCGGGCG ACTTCAGCGT CAAGCTGGGC GATTTCTCGA  
 1951 TGAACGGCAT CGGCACCGCC ACGTTCGGCG CCATCATCCT GTACGCCCTG  
 2001 CTGGGCCTGG CGCGTCGCCG CTGACGGCGC GCCAACCCGG CACCGGGGCC  
 2051 GCGCTCAGTG GGCGCGGGC GCGGGCAGGG CGGCATGGCC GCGCGCGGC  
 2101 CCGGCCAGCG GCGCGGGCG GACGTCGATG ATGGCGCCG CCTGCGTCT  
 2151 GAAGACCGCC GCCGCGGGC CCAGGTGGCG GGCCTGTTCT TCCATGGCCG  
 2201 TGGCCGCCG CGCGGCCTGT TCGACCAGCG CGGCATTCTG CTGGGTGGTT  
 2251 TCGTCCATTT GCGAAATCGC CAGGCTGACC TGGTCGATGC CGCTGGCCTG  
 2301 CTGGGCCGAG GCGGCCGAGA TCTCCCCCAT GATGTCGGCC ACGCGCTGTA  
 2351 CCGAGGCCAC CACCTCGTCC ATGGTGCCG CGGCGCTGGC GACCTGTTGC  
 2401 GAGCCGGCGC GCACGTCGC CACCGAGCTC TCGATCAGGG CCTTGATCTC  
 2451 CTTGGCCGCC TGCGCGGGC GCTGGGCCAG GCTGCGCACC TCGCCGCCA  
 2501 CCACCGCGAA GCCCTTGCCC TGTTCCGGG CGCGCGCCG CTCGACCGCG  
 2551 GCGTTCAGCG CCAGGATATT GGTCTGGAAG GCGATGCCGT CGATCACGGT  
 2601 GACGATGTCG GCGATCTGGC GCGAGCTCG GGAGATGCCG TGCATGGTCT  
 2651 GCACCACCTG CGCGACCGAC TCGCCGCCG GCTGCGCCAC CTGCATGCTG  
 2701 ACGGCGGCA GCTGATTGGC CTGCGGGCA TTGTCGGCGT TCTGCTTGAC  
 2751 CGTGGTGGCC AGTTCCTCCA TGGTGGCCG GGTCTCTTCC AGGGCGGCGG  
 2801 CCTGCTCCTC CGTGCGGCTG GACAGGTTGG CGTTGCCGGC CGAGATCTCG  
 2851 GCCGCGCCGA CGTTGATTTG GTCGACGCG CGCCGCATGA CCGCGATGGT  
 2901 GCGCGTCAGG CCTTCCTGCA TCGCTTGAG CGCCGCGAAC AGCSCGCCA  
 2951 TTTCAATTGGC CGAGCGCACC TCGATGCGCG CGGTGAGGTC GCCGTCGGCG  
 3001 ATGCGGTCGA AATGATGGCC GGCCTCCAGC AAGGGGCGCA GCACCGCGCG

Figure 5 (continued)

3051 GCGCACGAAC AGCCAGCCGG CCAGGGTCAG CAGCACGCCC AGCCTGGTCA  
3101 GCGCGATGGC GCTCCAGCGG GCCACGACAT GGGTGTCTCT GCGCCCGCTG  
3151 CGCACTTCGT CQCTGTGCGC CTGTACCCTG GCCAGAAAGG CTCCCATGTC  
3201 GCGCTGGAAT GCATGTTCCG CCTGCTGCGC GCGATGCATG GCGCCCAAGG  
3251 CGGGCTCGGC CTGGCCGCGG TCGACGGCGG CGGCCAGCTC GTCCAGCACC  
3301 GACTGGTAGG CGCGCCAGCG CGTCTGCAGG GTCGCGGCCA GCTCGGCCGC  
3351 GGCCGGCTCC TTGGGCACGT CCACGTAGCG CTGGAATTGC GTGGCCGCTT  
3401 GCGCTGCGC CGCGCCGCTG GCCTCGAACA GCGGGTCGAC CTGGTTGACG  
3451 GCGACCTGGT TCAGCCCCTC GATCTCGGCG GCGCTCCTGC CCSCGTTGCG  
3501 CCACGTCAGG GCGCCCGACA GCATCAGCAC CGCCAGGAAG CACCCGAATA  
3551 CCGCCACCAT CGCGGTGCGG ACCTTGATCC TTTCAAGCAT GGGCATCTCC  
3601 TGGGTAGGGT CTTGCGCAAT AAACGTGAC GACGTGCGCG GCGTACCCGC  
3651 GCGGGAAGTG TCAGGCCGTG ACGTGGAATC CGTTGTTGAT GTGGAATTCG  
3701 ACTTAAATGA TCGGTATTCT GTATTAATTT GTTATTGTGA GTTATATATA  
3751 CGAATATTCA TACCCGGATT TGCCCTAAGT TGGTGCCTTC TCGACGGGTG  
3801 CTTTCGATTG CCCGGGGCCT GCGGCGCATG AAGGAATGCG CGGCAACGCC  
3851 GGGCCCGCGC TCGCATCGGC GGCCAACACG CAGGTTTTGT GGCTTTTCCG  
3901 CAGCCAACAT GCGCCGAAAC CTACGCCGGG CTTACAGGCT TGCAATTCCG  
3951 GTGGACTTTG CCGACAATGT CATCTGATTG CCCCAGTTCC GACCCGAGCC  
4001 GGGGTTTTGT TTTGGTCGAC GCTTGGCCGC CGGATGCGGC AGGCCGATCA  
4051 AAGAGGAGAC AGCAAAGGGG AGCCTCGGTC GGGTTCGACC TTGTCTCGTC  
4101 TTTTGTTCGC CTGTCTTCCG CAGCGGCCCG CCTGTTTCAT GGCGAGGCCA  
4151 TACACCAAGC CGAGACCTTC ACCGAAACGC TCCGTCGGGG GCGTTTTCTA  
4201 CTTTTGTTTG GGAAACGACA TGTCTGCCAT TCCTTTGACC GTGCGCGGGG  
4251 CCGAGCGCTT GCAGCAAGAA CTGCATCGGC TTAAGACCGT TGAGCGTCCT  
4301 GCGGTGATCA GCGCCATTGC GGAGGCGCGT GCGCAGGGTG ATTTGTCGGA  
4351 AAATGCCGAG TACGACGCCG CCCGCGAACG CCAGGGCTTC ATCGAAGGCC  
4401 GGATCTCCGA ACTCGAGGCG ACGCTTTCGA ACGCGCACCT CATCGATCCA  
4451 ACGGCGCTCG ACGCCGAAGG CCGTGCCGTG TTCGGCGCGA CCGTGGAAAT  
4501 CGAAGACCTC GACTCGGGCG ACCGCCTGAC CTACCAGATC GTGGGCGACG

Figure 5 (continued)

4551 TCGAAGCCGA CATCAAGTCC AACCTGATTT CGGTCTCCAG CCCCTGGGC  
 4601 CGCGCCCTGA TCGGCAAATC CGAGGGCGAT GTGGTCGAAG TGAAGTGCC  
 4651 GGCTGGCGTG CGCGAGTACG AAGTCATCGG TGTGCGTTAT CTCTGACGCC  
 4701 GATTCCGCCC CCCTGCATAC CCATGGCCAA TGACCGACGC CTTTTCCACC  
 4751 ATCAGCCAGC CGCTTGCGGC CAGGCGGGGT GGCACAGCCG CAGCCGTCGC  
 4801 GCTGCAGGCG TCCCGGATGC GATATCCCGG GCTCCGGTCG TGGCCAGCGT  
 4851 ATTCCCCTTC TCGGCCGTCA ACGGCTTGGG GGGCGCGGTA TTCATCCGAA  
 4901 TTCCTGAACG CCGGGCCGTG CGCCGGGCGT CTGTATCGTT GTCGCGCCGC  
 4951 GATGCACGGA ACGTGCCGTC GGGCGCCGCA ACGCCGGGCC GCGCCGCCTA  
 5001 GGTGTGAACT GTCAATAGGT TGTATTCTGC CAGGTTGAGT CTGGAGATGG  
 5051 GTACAGCGCG CCCGATGCCT TGGTGGGGTC GATGCCAGTT GTAGTGGTGT  
 5101 AGCCAGGATT TCATGGCATC GGCTCGGTGT TGGGAGTTCT GGTAGGTGTG  
 5151 AGCGTAAGCC CACTCACGCA AGGCCGACTG GATGAAGCGT TCGGCCTTGC  
 5201 CATTGGTCTG TGGGCGGTAA GGTCCGGTAA AGCGGTGCTT GATGCCAGC  
 5251 TCATGGCACA GCGCGGCGAA GGCGCGGCTG CGAAAGGCCG AGCCATTGTC  
 5301 GGTGAGCAAG CGCTGGATGG TCACGCCAG GCGCTGGTAG TAGGCCACTG  
 5351 CGTCCTTGAG GAACTGGACG GCGCTGGGGA AGCGCTCGTC GGGGTGGATG  
 5401 TCGGTGAAGG CCACGCGGGC GTGGTCATCG ATGGCCACGA AGACGAAGTC  
 5451 CCAGCCGGCC CCCTCAACGG TATCGCGTCG GTTGCCCGTG ACCCGGTGGC  
 5501 CAGGGCGCTG GATACGTCCC AGCTTCTTGA TGTCGATGTG CAGCAGATCG  
 5551 CCGGGGGCCT GATGCTCGTA GCGCACCACC GGCTCGGCCG GCTCCAGGTC  
 5601 GGCCAGGTGC GACAGACCGG CGCGGGCCAG GACGCGGCTG ACGGTGCTGG  
 5651 CTGACACGCC CAGCGCCTGG GCGATGCGCG CTTGGGTCAG CCGCTTCCGG  
 5701 CGCAGCTCCA CGATAGCCAG CGCCTTGGCC GGC GCAATCG CTCGGGGCGA  
 5751 GACCGTCGGG CGCGAGGACG CATCGGCCAA GCCCGCCTGG CCCTGAGCCA  
 5801 GGAAGCGGCC CAGCCATTTG CGCACAGTCG GCGCGGTGAC CCCATAGGCG  
 5851 CGGGCCGCTT CAGGCACACA AACTTGATGG GCGATCAATT GCTGGACCAT  
 5901 TTCGAGTCGA CGTAGGAAGG TCAATCGGGC ATGCTTATGG GTGTTGATCC  
 5951 GGCCGGGCTC CTTGAGTGAA CTGGGGGGGT GGCGATTTC AGTTTCTCAA  
 6001 ATCCGGTTCG GATGAACCAT GCATACAACC TATTGAATCT TCACAACTAG  
 6051 CGCGCGTGGC GCGGAAAGAC CAGCAGGTCT GCCGTCACCG GTTCCCTGTT



Figure 5 (continued)

6101 GTCGAATAAC AGGTAATGCG TGGCGTCCGG ATATTGCGCA TGGCGCGCGC  
6151 CGGATCCGCA TGCCCTTGGC GGGGTCTCTG GCGCGGACGT CCGAAAACCG  
6201 GGATATTCCG AGAAGCAATC GGCTGGCGCC TTCCAGACTG TGCGCATACC  
6251 ATTGCCCTCT TTTGCCACGC ATTTGAGCG TATGGTTTCC CTTGCGCCCG  
6301 ACGCAGGCTC GCCTCGCCAT GACCGACACG GCATACCACC AACTCATCGC  
6351 CGATTTCTGGC CGCCTCATCG GCATCGACTC GCTCAACCCC GGTGCCGGCG  
6401 GCCTGTGTCA GTTGATTTTC GAACCGTGCG CACCGGTCTT CATCGCACCG  
6451 GTGCACGCCC GGACGGAAAT CATGATTTCC TGCCTGCTGG GCACGGCGGA  
6501 CGCGGCCAAC CCGGCAAGCA TGGCCCGAGC CAACTTCATG CAGGCCGGCA  
6551 GCGGCGTCGT GGCCTGCATC GCGGGCGATG GGTGTTCTA TCTGCAGCAG  
6601 GCCATACCCC TGTCGCGCGC CACGCCCGCA ATCCTGCTCG ATCACTGTGA  
6651 GCGTCTGTG CAGGAAGCCT CGCGCTGGCG CGTCGGCGAC CACGACGGCT  
6701 GCGCCACCTC GGCCCCGAAT ATCGCCGCGC TGACGCGCGG CGTCTAGGTC  
6751 GCGGCGGACT GCGGGGCGCC GCGCGGCGAG GCTCAACTCG CTTCTGTAT  
6801 GACGCCCTTG AGCGAATCCG CGACCTGCTT GACCACCGTG CTCTGTAGAT  
6851 CGATCATCAC GACCCACGAT TGCATTTCTT GTTGCACGAT CAGCAGGTCG  
6901 GACGTGCTGA CCGCGCCGTC TCCGCGCGCG CTGAGCGCCT CCAGGCGGCT  
6951 GCGCAGGTCG CGTTCGTGAG CGTTCAGCCG CGTATTGACC GCCTGGTTGA  
7001 CGCTCTGCAT GGTCACTCGG CCTGCGTCGC CTCCCAGGTT AATGGCCATG  
7051 CTTGTCTCCT TCGGCGCATT GTTCATTGCT CAGGCGCGTC AAGACTGACG  
7101 CCGGAGGGTT GTCCGGCCCG GTCGGGCGCT GCAGCAATAC CTGCCGGGCC  
7151 GCGGTGGCGG CCGCCAGCGC GTCGCGCCAT ACGGGATAGG TGTCGCGCCC  
7201 CACGCCATCG TGCAGGCGGA CGCGCAAACG TGTCTCCAGT TCGCCAAGCT  
7251 GCGACAGCAA GGTGTGCGCG AAGGCGGAAC CGCCCGGCGA TGCCAGGCGC  
7301 ACTTCCAGTT CGGTGAGGGC TAGAACAGAT GTACTCATAT GATGTTGCAG  
7351 ACGAGGGTTG ACGGCTACCG AGGCGATTTC ATCGCGTCAC GATGACCGGT  
7401 TCGGGACCAT CGAAGACCAG GCGGCCGAT TCGATGGCGG TAAGGCGATA  
7451 CTGGTCCCGC AGTCCGCCCA CCAGGAGGCG GCTGCCATCG GCCAGCATCA  
7501 GGTACGGTTG CGGGCGGCTC ACGACACTGC GTATCTCGAA CGGCACGTGA  
7551 TCCCGCGTCG CGCGCGCGG GGTGGCCGGC AGCCGGACGA CGTCGTAGTT

Figure 5 (continued)

7601 GCGCTGGTTG AACGCGGCCA CCAGCTCGCG CAGGCGCGCC ATGCGGCCTG  
 7651 CCGCCAATCC GCCGGGATCT GCGTCCAGGC GGTGGGCGTG CCAGCTGAGC  
 7701 TTGACGCCGT CGAGGCGTTC GTCGGCCAGC TGGGCCGCGA ACTGGGCCGA  
 7751 GACCTCGTCG GCCAGGCGTA CATCGCGACC GAGGATCGTC ATGCCCCGCA  
 7801 GCGCATGCG CACCGCATGC AGCGCCGCGG CGCGTTCGTG CGCATCGCTG  
 7851 GCGATGCCCC AGATCGCCAG GCGGCCATTG CCGTACGGGC GCGCCATGTA  
 7901 GCGCACCCCG AATGTCGCCA GGACATCGCA GGCCAGGGCC CTGGCCTCGT  
 7951 CCTGCCTGCT TACCTGCATG GCAGGCCGTG GCGCAAGTTG CGCCAACGCC  
 8001 CTGGCGACCC GAGCGAATTC CGTCTCGTCG TGCACCCATC CGGTCACGGT  
 8051 GAGCACGCCG CCACGGCCGT AGGCCGCTTG TAATTGCTCG GTAAGGCCCA  
 8101 GGCTGTGAT GAGCGCCCG GCGCGGACCA GCGGCGCGGT GGGCGTTGGG  
 8151 GGCGGCGCGG CCGGCGCGGT GCGGGGTGTG GTCACGAAA CCAGCGCCGT  
 8201 GGCCAGGCCG ACCAGCAGGA CGGCCGCGGC CGCGCCAGC GCCAGCCAGG  
 8251 GCCGTCCTGC ACGTCGGCGC GGCATGAGGG CAGCGACGGA CGCGGGGCTT  
 8301 GTCGAGCCAG GGACGTCGTG CAAGGCTGTG TCGCTGCCGT CCGGGCCGCA  
 8351 CGGCTCCGGC GCGCGGGGCC ACGGCGCGGA AGGGGCGGCC ACGGTGATCC  
 8401 AGGCGGCTCC CAGCTCTACG GGTTCGTTGA AGGCCGCGGG CGGACACGGC  
 8451 GCCTGGGCGT CCAGGCCGGG CGTCACGGCG CCGGCCAACC GCCAGCCGGA  
 8501 CTGGTCGATC TCCAGCCATC CCGCCACTTC GGGCATGTCC TCGCCGGTCA  
 8551 GGACGATATC GCAATGCGGA TTGGCGCCA CGCGCGCGCC ATGCACGGCC  
 8601 GGGCAGCGCG CCATGCACTG TGCGCCTGAA AGCACGCGGA ATTCCAGCGC  
 8651 CGTCGTCATA GATCCACCCT GCCAGGGGC TGTACATTGA TCTCCGGCGT  
 8701 CAGTTCCTGG TAGGACAGCA CCGGCAGGGC GTAGAGATCG GCTTCTATCA  
 8751 TCTTGCGCGT GTAGCGCCGG ATGTCCATCG ACGTCAGCAA GACGGGACGG  
 8801 CTCGCGCCGG CGGCCAGATC GCCGACACAT TGACGGATGT GCTCGACCAG  
 8851 TCGGCGTGTC GTGTCCGGAT CGAGGGCGAG ATAAGTCCG GCGCGGTCT  
 8901 GCCGGATGGC GCGCGCACG GTTTCCTCGA CCTTGGGGGC CAGCAGGTAG  
 8951 GCGGGCAGGA TATTGTGGCC GCTGGTGTAC TTGTGGCTGA TATAGCGCTT  
 9001 GAGTGCGATT CGGACATACT CCGTAAGCAG GACGGTATCC TTTTCCTTCT  
 9051 GGCCCCATTC GACCAGCGCT TCCAGGACGG CGCGCAGGTT GCGTATCGAC  
 9101 ACTTCTTCGG AAACAAGGCG CTGCAGGATT TCGGCAATCT TCTGCACCGG

Figure 5 (continued)

9151 CATGACGCGC AGGCACTCCT TGACCAGATC GGGAAATCGT TCTTCCATGG  
 9201 CCGAAAGCAG AAACCGGGTT TCCTGGATGC CGATGAAATC GGCTGAATAT  
 9251 TTTTTCATAA CATATGCCAA GTGCCAAGTC AGGATCTGGC TGATACCCAG  
 9301 GTAAGGAATA CCTGCATCGC GCAAGGCGCC GGTCAGACTG GCCGCAACCC  
 9351 AGATCGTGGG CGTATCGGGC AGAAAGGCCG CGCCCGTTTC GTATGCGATC  
 9401 CGCAGGGCCT GCAGGTTCTG CTCGGTGTCC CGCACCAGCA CGGCATCGTC  
 9451 GCGCAACATT CCTTGCGCCA CCGGGATCTC CGACAGCACG ATGSGTAGG  
 9501 TATTGGCGGC CAGCGCTTCG GTGAAGCGCA ACTGGATGCC GGGAAACGGC  
 9551 ACGCCCAGGT CGAAATAGAG CGCCCGCCGG ATCTGCAGCA GATCGTCGGT  
 9601 GAGGGTGGCC GGCTCGAACC GGGGCTGCAG CCGCGCGGCT ACGTCGATGA  
 9651 TCAGCGGGAC GGTGGGGGCG AATTCCGCCT GCCCATCCGC CGGCGCGCGG  
 9701 GTGCGGGGCT GGCCGTCGGC AGCCATGCCG GCGAGCGCGG GCTCGGCGCC  
 9751 TTCGGGCGGA CGCTGGGATG CGCGCAGCAG TACGAAACCG ATGGTGCCCA  
 9801 CCGCGGCGGC CAGGGCGAAG AAGACCAGCG TGGGCATGCC GGGAAATGAGG  
 9851 CCCAGGCCTG CCGAGATCGC GCCGGCAATG ACCAGGGCGC GAGGCTGCGC  
 9901 CAGCACTTGT GCGCCGATGT CGGTGCCTAC GTTGGAGGGG CCATCCCCGG  
 9951 TCTGCACCCG CGTCACGATG ATCCGGCGC AGATGGCGAT GAACAGCGCC  
 10001 GGGATCTGCG CGATGAGCCC GTCGCCTATG GTCAGGATGG CATATGTCTG  
 10051 CACGGCCTCG CCGGCGCTCA GGCCGCGCTG CAGCACGCCG ACCAGCATGC  
 10101 CGCCAAGCAG GTTGACGGCA ACGATGATCA GGCCGGCGAT GGCATCGCCC  
 10151 TTGACGAACT TCATCGCGCC GTCCATGGCG CCATACAGTT GCCTTTCCTT  
 10201 CTCGACCGTA CGGCGTCGGC GTCGGGCTTC GTCCATGTCT ATGGTGCCCG  
 10251 CGCGCAAGTC CGCGTCGATG GACATCTGCT TGCCGGGCAT GGCCTCCAGC  
 10301 GAGAAGCGCG CGGCGACTTC GGCCACCCGC TCCGCGCCTT TGGTGATGAC  
 10351 CACGAACTGC ACGATCGTGA GGATGAGGAA AACCACCAGG CCGACGATCA  
 10401 GGTTGCCGCC CACCACGAAG TTGCCGAAGG TCTCGATGAT GTGGCCGGCA  
 10451 TCGCCTTGCA GCAGGATCAG CCGCGTGGTC GCGATGGAGA TGCCAGCCG  
 10501 GAACAGCGTG GTGACCAGCA GGACCGAAGG GAACGAGGAA AACGCCAGGG  
 10551 GCGAAGGCAG GTACATCGCG ACCATCAGCA GGAAGTCCGA CAGGTCATG  
 10601 TTCGCACCGA TCAGCACGTC GACCAGCGTT GTGGGCAACG GCAGGATCAT

Figure 5 (continued)

10651 CATGAAGACG ATCGCCACGA TGAGCACGGC CAGTACGATG TCGTTGCCGC  
 10701 TGGTGGCCAG CGCCACCGCG CGTTGCAGGC GCGAATGGA TTTCTTGCTC  
 10751 GTCATGGCGG GGTGCGCTC GCGCAAGACG CCGCCCGTAG CGCCATATAG  
 10801 TCACGCATGG CCTGGCGGGC GTCGTGCGGT CGGTTGAGCG CCTGCATGGC  
 10851 CTGCGCCCGG ACCAGATGGC CGGCGGCATC GGGTGTGGCG CGCAGTGGC  
 10901 GCTTGTCCAG CGTGACCAAG GCCATGCGCG GTTCGCCCTG GTGCAGATAG  
 10951 CCCAGCGCCA GGGCCAGCAG GGAAGTGGCTG TCGATCGCGT CCAGGGCATC  
 11001 CAGGGCCGCC AGCAGGGCAA CCGTCTTGCT CCATTGGCGC TGCAACTGGT  
 11051 AGTGGTGGCG AAGCAATTGC AGAAGTTCGC GTACCTGAAG GCTGGGCGAG  
 11101 GGTAGGGTAT GCGGCATCAT CCCTGGTAGA GCGCGCTGCG ATACATGGCA  
 11151 GCCAGGTGCG GCAGCTTTCC GGCTTCGTTG AGTACGTGCA AGGCGCGGCT  
 11201 TAAGGCTGGC GCGGTGTTGC CGGCATGCAG TTCCATGGCC CGGGACAGTT  
 11251 CGTCGCGCGC CTGGGCCAGG GCGCGCTCGA ACTGCGCGGG CTGGAATAAT  
 11301 GCGCCATCCG TCAGGCGAGG CCGCGCCGAC TCGTCGAGCA TCGCGCTCG  
 11351 GTCCGGGCGT ACAGAGAGGG CTTTCAGATG ATCGACCGCG CCGGTGGCGG  
 11401 GCGGTTCGAG CCAGCGCTCG GGTGGCAGGG TGGGGGCGGG CTCGCAGCGG  
 11451 GGACCGCGCA CGATGTGGTC GACGCCGCGC TCCAGGCCGA GGTGCATGGC  
 11501 ATGCATGCCG GGTACGGCGC TGGACATGGC GTCACGCCTC CAGGCGGTGC  
 11551 AGCCAGTTCG TCAGGCCAG CACCGCCAGG CGCAACGCCG CTGCGTCGAG  
 11601 CGAGCGTTCG GGCAGTCGTG TCTGCGCGAC GATCCAGTCC TCGCTGCCCT  
 11651 CCGACCAGAG TGACGTCTGG ATGGATGCGG CGCTTCCGCG CTGCCCATGG  
 11701 GCCCCTTTCC ATGCCGCCAG CAACACGGAG GCGGCGTCGC GCTCGACCCG  
 11751 CTGGGCCAGG TGGACCAGGG CCGCGCCGGC GACGCATTCG ACGCCCAGGC  
 11801 GCGCCCCGTT GGACAGCGCC AGCGACGCCG ATCCGGACGG CCCGAATGCC  
 11851 AGGCCCTCGA TGCCGATATC CTGGCCGAAC TGATGCAGCG CCCTATCGGC  
 11901 AGTATTCATG CGTTCTCCAT TGCTATCGCG TTGTCCAGCG CGTCTGCGC  
 11951 GGCCGCCAGG ACGGTGGCGC GCACGTCCAT GTCGGCGTAG ATCTGCGTGG  
 12001 GCAGGTCTTT GAGAATCTGG CGTACGCCAC CGAGGAATGC GATGCGCTCG  
 12051 GAGAGGGCAT TCGCACCGTG GCGCTCGGCC AGCTTCTCGA AGCGCGCGGG  
 12101 CGCAATCCAT TTGTCTCGC TGATTCCCAC AAGATCGCGC ATCAGGCCCT  
 12151 GGGCGTCCGC AACTCCTGC GAGCCTGCGT TGCCCAACCG TTGTTTCAGG

Figure 5 (continued)

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12201 GCATTGCATT CCTCCAGTAC CGTGGCGGCC ACCTCGACTT GATAGAGATC
12251 GCTCGCCAAC ACTTGACAGCC TGACGCCGTC CGTCGACGGT GTGGCGCGGG
12301 CCAGGTCGTG TCCCAGCGCC TGAATCAGCG CGCCCAGCGC GCCGTGGATG
12351 TCGTCGTTCC CATAGCGTTC CAGCACCAGG TCCAGCGTGC GCGCCAAACG
12401 CAGCTGGCCC AGGGCGATGT CGCGGTACGC GTGCTGGAAG CCGGCCAGCT
12451 CGTCAGCGGA ACGCGCGAAT GCGCCGGCCG TGGGCAGGGT GTTGATGCCG
12501 GCGCGGATTT CGGGGCCATG GCGAGCTCC AGGTCGGCCA ATGCATCCCG
12551 CAGGGCTTCG AGCGCGTGCG GCGCGGCGTC CTCGTGCTCG CCGCGCTGCA
12601 GCGCGTGCTG CAGCGCGAGG TATTGCTGCG TGACACCGGG AAACGCTTGC
12651 GCGGCCAGCT GCATGGGGGC GCGCGGCGC CGCAGCAGCT CCGCGGTCAG
12701 GGCTTCCAGT TTTGCCTGCG CGTCGGGGTC GTGGGTGTGG GAAAACAGTT
12751 CCGCAAGCTG CGCCGCGTCC AGCCAGAGCA TCGGACGTTT GGCCGTGACC
12801 TTGCGTTCGG AGTGATGCTT TTCCTCGGCA GCCTGCGCCA TGTGCAGGCT
12851 GAGCTCCTCG GCCGCGTCCG CCAGCGATAT GCCGGTGGGC GCCGGTGCGA
12901 TGCGCTGGCC TTGCAGCCAG CCGGAGGAGG TGTTGGCCGA GGCGTCGTGG
12951 CGCCCCTGCA TGGCGGCGTG GAAGGGATTG GGGGCGGCAT CGATACGACT
13001 CATGGGGAGT CCTCGGAGAA GGAACCATTT GCCTACTGGT GCAGTGAGTG
13051 TCGCGCGCGC GGTTCATGGTT CCCGGAAACG GCGCGATAT TGGGCAATTC
13101 GCAGCCTGGA ACTTGCCGCG GCGCGAGGGT TACTCAGCAT GCGTCTTTCA
13151 ACTCGAAGGA GCTCTCATGA GCATTGATCT CGGAGTTTCA CTCACGTGCG
13201 AGGCCGGCGG CCTGCAAGGC ATCGACCTCA AGAGCATGGA TATCCAGACT
13251 CTCATGGTGT ATGTGCAGGG TCGTCGCGCC GAACTCCTCA CGGCTCAAT
13301 GCAGACCCAG GCCGAAGTGG TGCAGAAGGC CAATGAACGC ATGGCGCAGC
13351 TCAACGAGGT CCTGTCCGCG CTGTCCCGGG CCAAGGCCGA GTTTCCGGCC
13401 AATCCGAAGC CGGGCGACAC CATCCCGGGC TGGGACAGCC AGAAGATCAG
13451 CCGGATCGAG GTTCTCTCTA ATGATGCGCT GCGTGCCGCC GGCCTGACGG
13501 GCATGTTCTG AGCGCGCGAT GGCCGGGTGA CCGGCCCCGA CGGCCGGGGT
13551 ACGCAGSTCG TGAACGGCAC GGGCGTCATG GCCGGTTCCA CGACCTATTA
13601 GGAACTCGAA AGTGCCTACA CCACCGTAAA GGGGATGCTG GATACGGCGT
13651 CCAATACGCA ACAGATGGAC ATGATCAGGC TGCAGGCCGC CAGCACCAAG

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Figure 5 (continued)

13701 CGCAACGAGG CTTTCGAGGT CATGACCAAC ACCGAGAAGC GGCGCAGCGA  
 13751 CTTGAACAGC TCCATCACCA GCAACATGCG CTAAGCGCTG CACAAGGAGT  
 13801 ATTCCATGCA GGAGCAAGGC ATCCAATCCA TCATGCGCGC CGCGGAAGAG  
 13851 CTGGTCGAGC AGACCCGCCA GGCCTTGTAC AGCGTCGACG AGATCTACGC  
 13901 CCACGTTGGC GTCGACCCCG CTCGCCTGCG CAATCTGGCG GTCCAGCAGG  
 13951 CCAGGATAGA GGCCGAGGCC CAGGCGGCGT TCCGTGATGA CCTCGCGGAC  
 14001 ATCGAGCGCG AGGCGGCGCG CGTCAAGGCG GCCTGCACCG ATGC3CCGCA  
 14051 GGCCCGCAGG GTGCTTCACA ACCACGTCTG AGCGCGGAGG CCTTCCATGC  
 14101 CAAAGTCAGC CGACCAGGGC GGCTCCCCGG CGTCAGCTTC GCATGAGGCG  
 14151 TTGCGCCATA TTCTCGACGC AGGCGCTTCG ATGGGGGGCT TGCAGGGGTT  
 14201 GGACGAGGCG CAGCAGCAGG CGTTGTACGC GATCGGTCAT GGCGCTACG  
 14251 AACAGGGGCG CTATGCCGAC GCGTTGAAAA TGTTCTGCCT GCTGGTCGCG  
 14301 TCGGATCCGC TGGAAGCCCG TTATCTGCTG GCCCTGGGCG CCGCGGCCCA  
 14351 GGAGCTGGGG CTGTACGAGC ATGCCTTGCA GCAATACGCG GCCGCGGCGG  
 14401 CTTTGCAGTT GGAATCCCCC AGGCCCCCTGT TGCATGGCGC CGAGTGCCTG  
 14451 TATGCGTTGG GTCGTCGCGG CGACGCCCTG GATACGCTCG ACATGGTGCT  
 14501 TGAGTTGTGC GGCTCGCCGG AGCGTGCGGC CCTGCGCGAA CGGGCCGAGT  
 14551 TGCTGCGCAG GAGCTATGCA CGTGCCGACT GAAACGGCGC CATGTCCGCC  
 14601 GTCAAGATTT CAATTCGAGG AGGTTGATA TGTCTGTTTC TCCGACTTCG  
 14651 CCCGGCTCTT TCGGGGCCGG CCCTGTCTTT GACTCCGAAT TGCAGGCCCC  
 14701 GGCCCCGTG GCGCAGCGTC GCGGCGGTGC GGCCTGTG CCGCCGCCCG  
 14751 TCGATCGGCG CGGCGTCGAG CCGGGAGATC CCACGCTGGG CATGCTGCCC  
 14801 GCGCCAGATT TGCTCGCGGG GGGCGCCGTC AGCCGCACCC GCGCGGCGCT  
 14851 CGACGATCTG GACGCCGCAC GGCTCGGTGA AGACATCTAC GCCTTGATGG  
 14901 CCGTGTGCA ACAGGCCAGT CAGCAGATGC GGGACGCCGC CCGTATCGCT  
 14951 CGTGATGCCG AGGCTACGCG GCAAACGCAG GCTCTCGGCG ATGCGGCCAG  
 15001 CCAGATGCGC CAGGCGGCGA GCGAGCGCAT GGCCGGAGCG ATCGTGCCGG  
 15051 GCGCCATGCA GATAGCGGGT GGTTCGTGC AGCTGGGGGC GGGCTGGCA  
 15101 GCGGGTTTGC AGGCCATGGG TGGCGCTGCT GCGCAAGCCA AGGGCGCCGC  
 15151 ATTCTCCGAG CAGGCCTCGA CAAGCCGCAA GGTGGCGGCC GGCTTGACAG  
 15201 ATGCCCCCGA GCTGCAGGCA ACGGTGCAGG CCCGCGCAAC CCAGCTCGAA

Figure 5 (continued)

15251 GCGCAAGCGG CCTCGTTTGG TCGGACGCG GCTCGTTCGT CGGCAAGTC  
 15301 GCAGCGCGTA TCGAGCGTTG CCCAGGCCGG CGCCGCAGCG GCCGGCGGTA  
 15351 TCGGCGGCCT GACCAGCGCC GCCCAGGAAC GCCGCGCCGC CGAGCACGAG  
 15401 GCCAGGCGCG CGGAGCTGGA CGTCGAAGCG AAGGTGCATG AAGCGGCCTC  
 15451 GCGGCGGGCC GACGAAGCCA TGCAGCAGAT GCTCGACATC ATCCGCGGCA  
 15501 TCAGGGAAAA GCTGGCCGGG ATGGAGCAGT CCCGCAGCGA GACCGCCCT  
 15551 AGCGTGGCCC GCAATATCTG AGTGTCCGGC TCCAACCTTC AATCTTGAGG  
 15601 ATGACCGTCA TGAGTACGAC CATATCCACA GCCCCGAGCG GCGCCGCGCT  
 15651 TGCGCCGTCT CGCATAGATA TCGGGGCGCC GGAGCCCGGG AGTGCCGCGG  
 15701 AAGGCGCCGG TATCCTGGCG CCGGTGACGA CGCTGGCTCT GCGGCGGGG  
 15751 CGGCCGGCTT TGCCAGCGTC ACCGTGCGTG CGCACCGCGC CCGTCTGGA  
 15801 TCCGCCAGTG CGCGATCTCA GCCCCGCCGA CTTGGCCGAC CTGCTGCGCG  
 15851 TCTTGCGATC CAGGGCGGTG GACGGGCGAG TGGCCACGGC GCGCGAGAC  
 15901 CTGCAGGATG CGCAAGTCAA GGCGAAGCAG AACACCCAGG CCCAGCTCGA  
 15951 CAAGCTGGAC GCATGGTTTC GGAAGGCTGA GGACGCCGAG AGCAAGGGCT  
 16001 GGCTGAGCAA GGTGTTCCGG TGGATCGGGA AGGTGCTGGC GGTGCTGGCA  
 16051 TCGGCCCTGG CTGTGGGCTT TGCTGCCGTC GCCAGCGTGG TCACCGGCGC  
 16101 GGCGGCCACG CCCATGCTGG TGCTCAGCGG CATGGCATTG GTCAGCGCCG  
 16151 TGACATCGCT GGCCGACCAG ATATCGCGAG AGGCGGGAGG GCCGCTATC  
 16201 AGCCTGGGCG GGTTCCTCTC CGGGCTGGCC GGACGTCTGC TGACAGCGTT  
 16251 GGGGGTGGAT CAGTCGAGG CCGACCAAAT TGCCAAGATC GTCGCGGCC  
 16301 TGGCCGTGCC CGCCGTCTTG CTGATCGAAC CCCAGATGCT GGGCGAAATG  
 16351 GCCGAAGGCG TGGCCAGGCT GGCGGGCGCC GGCGATGCCA CCGCGGGATA  
 16401 CATAGCCATG GCGATGTCCA TCGTGGCGGC GATCGCGGTC GCGCGATCA  
 16451 ATGCCGCCGG TACGGCCGGC GCGGGCAGCG CCTCGGCGAT CAGGGGTGCG  
 16501 TGGGATCGGG CCGCCGCGGT AGCCACCCAG GTCCTTCAGG GGGGTACGGC  
 16551 AGTGGCGCAA GGCGGCGTCG GCGTGTCGAT GGCAGTCGAT CGCAACAGG  
 16601 CCGATCTCCT GGTGCGCGAC AAGGCGGATC TGGCGGCGAG CCTGACAAA  
 16651 CTGCGGGCGG CCATGGAGCG TGAGGCGGAC GATATCAAGA AGATCCTGGC  
 16701 TCAATTCGAC GCGGCCTATC ACATGATCGC GCAGATGATC AGCGACATGG

Figure 5 (continued)

16751 CGAGCACGCA CAGCCAGGTC AGCGCCAACC TCGGACGGCG CCAGGCGGTG  
16801 TAGCGCCGGG CGCTCAAGGA ATTTTCATGA CTGTTTCATGA CGACGCGGGC  
16851 GCGGCGCTGC GCGCCCGGCT GGATGCGTTG CCGGGCAGCC GCGGCTGAC  
16901 AGCCGAGCAA TTGGAAGTGA TTTACGCGAT GGCGTATGCG CAGCTCGCCA  
16951 GGTGCGAGTA CGGCAAGGCG CTGCCCCATT TCGCCTTCCT CGGCGAGTAC  
17001 GGCCCCACGC GCAAGCACTA CTGGGCCGGC CTGGCGCTAT GCCTGCAGAA  
17051 GACCGACCGT CCCGACGAGG CGCGCAATAT CTATGCGTTG ATCCTCACGC  
17101 TCTATCCAGA TTCCGCGGAT GCCGTGTTGC GCACGGCCGA GTGCGAGCTG  
17151 GCGTTGGGTG AGAACGAACG GCGCGAGGCG GCCCTGTTCG GCGCAATCGC  
17201 CATCGATGCA GAAAGTGGGC AGCCAGGTCC GGTCTCGCAC CGTGGCGCGC  
17251 CTTTGCTCGA TCTTATTTCA GTTTCACATC CGGAGTAACT CCATGCACTC  
17301 AGACTCAGGT TCAGATTCAG GCTCAGACTC AGGCTCAGGC TCACCCATGG  
17351 TCTCGTCGAT ACATCCATCG GAACCGATAC AGCCGATGGA GCATGTGCTC  
17401 GAGGAGGCCG ACGCCCGCCT GCTTACCGAA GTGGGTTTTC TGGCGGCGGC  
17451 CGTCAGCGAT CTGACGCGCG CGGACGCCAT TTTCAATGCA TTGCAACGTG  
17501 TACGGCCGGG CCGGACGCAT CCCTGCATCG GCCTGGCGGT CGCCCGCATG  
17551 AACGCCGGGC TGCCCGACGA AGCCGCCGAG ATCCTGGCGA ATTTCCAGCC  
17601 GGCACAGCCG GAGGACCGCT CGGAAGTGA GGCCTGGTGC GGGTTCGCTC  
17651 TGTTGCTGGC TGGCCGCTCG GACGAGGCGC GCCGCATGCT GCAGCGAGCC  
17701 ATCGATGCGG GTGGCGAGGC GGCAAGGCTG GCGCAGGTCG TGTGGACAG  
17751 CGGACCCGCC ATGATGCGGC CCGCGCCGTT GCAGTCCGAG CCATTACCTG  
17801 GAGCTCCTGG ATGAATTTGG ATCTGACGGC GATCAACGCC GTGCAGGAAC  
17851 GGCTGCTCGC TGGATCATTC GACATGCCGC GATCTCCCGC GATGGCGGAT  
17901 CAGGCGCGCT TTGAATTGGC GCTGGGCGAG ATGCCCCGCG CATCGGCCCC  
17951 GAACGGGGCG ATCGCCCTGG CGCCGGTTCG GCTCGACGAG CCGCTGGGCC  
18001 GTCGCATTCT TGGACAGTTG CGCGGCGGCC TGGCCGATGT GGCAGGAAA  
18051 TGGCGGGCGG TGCAGACGGG CTTGGCCGAG GTGAGCCAGG CGCCTACCGT  
18101 GGTGGGTATG CTCGATCTGC AGGCCAGGTT GCTACAGGCA TCCGTGGAGT  
18151 ACGAGTTGGT GGGCAAGGCA ATAGGGCGCG CCACCCAAAA CGTCGATACG  
18201 CTGGCGAGAA TGTCAATGAAC GCCATCGGGG CGATCCAACG GTATCGGGCC  
18251 GGCGCGGGAT GGGCGGCCCT GGTGCTCGCC CTGGCGCTGC TGGCCGGCTG



Figure 5 (continued)

18301 CGGTGCCCCG GTCGAGCTGT TGGGCGCGGC GCCCGAGAAC GAAGCCAAAG  
18351 AAGTATTGGC GGCCTGTCTC GAGGCAGGCA TCGCTGCGCA GAAGCAGTCC  
18401 GGCAAGGCCG GCTACGCGGT TTCGGTGCCG GCCGAGGCGG TGGCCCGGTC  
18451 GCTGGAGATC CTGCGCGCAA GCGGCCTGCC CCGCGAGCAG TTGACGGAA  
18501 TGGGACGCAT ATTCCGCAAG GAAGGCCTGG TTTCATCTCC GCTCGAAGAG  
18551 CGCGCCCGCT ACATTTATGC GCTGTCTCAG GAATTGGCCG ACACCCCTGC  
18601 GCAGATCGAC GGCCTGTCTA GCGCCCGCGT GCACGTGGTG CTCCCGAAC  
18651 GCGGCGCGGT CGGCGAGCCG GCCACCCCTT CGACGGCAGG GGTGTTTCTC  
18701 AAGTACCGCG ACGGACAGAG CCTCGACGCG CTCGTGCCCG AGATCCGCAA  
18751 GCTGGTCACG CATGCCATCC CGGGCCTGGC CGAGGACCGT GTATCGGTG  
18801 CCCTGGTGGT GGCCAGCCC GTTCAGGCCG CACCCGCGCC GGTGCGGTG  
18851 CGCCGCGTGC TTGGCTACA GGTCGCGGAC GGATCGGTCC TGAGATTTTC  
18901 GCTGTTGCTG CTGTTGTTGC CGGTGCTGTG CCTGATAGTG GCGGGGGCCG  
18951 CGCTCTACGT CTGGCGCACG CGCTGGTCCC GCGGCGAAGG GCGCGGCGGC  
19001 GCTGGCGCCG GCGCCACGGA AGGAGCCGGG CATGACTGAG GCGAGCGTGC  
19051 TGCTTTCCGA GCGGCTCATG ATATTCAATC TCCTGCCAG CCTGACCCTG  
19101 CATGCCAGTC GCCACGACGA GATGTTTCCA GCCGATTGGG TGCCGCGTTC  
19151 GTGCAATGCC GACGCGGCGT TGGCCAACGC GTGGCATCGC CATTGGTCCG  
19201 GCTGGATCTT GTGCGAATTG GGCCTGCTGA ACCAGCCGGT CCTGAGCCTC  
19251 GATCCGCCGC AGTTGAAGGT CGCGCTATTG TCCACGGACG CCTTGCGGAC  
19301 CTGCGCCGCC CATGCGGGAG CGCTGCTGTG CGCGCCGCGC CTGCGACGCG  
19351 CGATAGACGG CGCCGAGGTC CGTACCTTGC ATGCCGCGCT CGGGCGCGAT  
19401 GTGATGAATT TCGCCGTGTC TTCCGCGGCG CGGGCCCTGC ATGACGGGCT  
19451 CGCCGCCAGT TCGGACTGGA CCCTGGCCGC CACGGTCCAG GCGGCGCAGA  
19501 AACTGGGCTG GGCCCTGCTG CGCGACGCCG TGCAGGGCGC CGCCGACGAG  
19551 ATAGCGCTGC GTTGCGCGCT GAAGTTGCCG CGCGACCTTG ATCCGCGGCC  
19601 CGTCTGCGG CCCGAGGCGG CGCTTGCCTT GGTGCTGTCC ATGCTCGAAA  
19651 TCCTGGATGC AGAATGGCTT TCCTCGTTCC CCGCCCAAGC CTGATCCAGG  
19701 CGGTACGGCC CGGCCGTGCG GATCCCGCGA CCGACGTCTT GCGCGCTGAA  
19751 GACTACGCCG AGCTGCTCAG CGCCGCGCAG ATCGTTGCCC AGGCACACTG

Figure 5 (continued)

19801 GCGGGCCGGC GAAATCGTGG CCGAGGCGCG AGAGGAGTTC GAGCGCGAGC  
19851 GCAGGCGAGG CTATGAGGAG GGGCGCCGCG AAGCGCTTAC GGATCAGGCG  
19901 GAGAAGATGA TAGAAACCGT AAGCCGCACG ATCGACTACT TCGCGGGTAT  
19951 CGAGAACGAG ATGATCGAAC TGGTCATGAG TGCGGTCCGC AAGATCGTCG  
20001 ACGGTTACGA CGACCGCGAG CGCACCCTGA TCGCCGTGCG CAACGCATTG  
20051 GCGGTCTGTC GCAATCAGCG CCAGATGACC TTGCGCCTGC ACCCAGACGA  
20101 GGTGGATGTG CTCCGGGAAG GCATGAACCA GCTTCTGGCG GCCTATCCGG  
20151 GCGTGGGCTA CCTGGACCTG CTGCCCAGCG CCAGGCTGGC GCCGGGAGCC  
20201 TGCATACTGG AGAGCGAGAT AGGCATGGTC GAGGCCAGCC TCGAGGACCA  
20251 GCTGTGCGCC TTGCGGGCGG CCTTCGAACG TACATTCGGC CGGCGCGGAT  
20301 AGGGGCATGC GTCAGTACCA CTACATCACG GAGATGATGC GGGTGGCCCT  
20351 GCAGGATCTG TCCACGCTGC GGATAAAGGG CCGGGTGGTG CAAGTGGTGG  
20401 GAACGATCAT CAAGGCCGTC GTTCCGATGG TCAAGATCGG CGAAGTGTGC  
20451 CTGCTGCGCA ATCCCAGCGA GGAATTCGAG ATGCACGGCG AAGTGGTGGG  
20501 CTTTGTCCGC GACGCCGCCT TGCTCACGCC TATCGGCGAC ATGTACGGGA  
20551 TTTCTTCGGC GACCGAGGTG ATACCGACCG GACGCACGCA TATGGTCCCC  
20601 GTCGGTCCGG GCTTGCTGGG ACGCGTGCTG GACGGGCTGG GACGTCCGCT  
20651 GGACGCCGCC GAGTCAGGGC CGCTGCATGC CCACAAGTTC TATCCGGTCT  
20701 TCGCCGATGC GCCAGACCCG CTGACGCGTC GCATCATCCA TGCTCCGCTG  
20751 GAGCTGGGGG TGCGCGTACT GGACGGTTTG CTTACATGCG GGGPAGGCCA  
20801 GCGTCTGGGA ATTTTCGCAG CCGCCGGCGG CGGCAAGTCG ACCCTGCTGG  
20851 GCATGCTGGT CAAGGGCGCC GCGGTCGACG TGACGGTGGT GGCGCTGATC  
20901 GGCGAGCGTG GGCGGGAAGT TCGCGAGTTC CTTGAGCACG AACTCGGTCC  
20951 GGAGGGCAGA CGCAAGAGCG TGATCGTCTG CGCGACCAGC GACAAGTCCT  
21001 CGATGGAGCG TGCCAAGGCG GCGTACGTCG CAACCGCCAT CGCCGAATAC  
21051 TTCCGCGATC AAGGGCAGCG TGTACTTTTT CTGATGGACT CGGTACCCCG  
21101 CTTTGCGCGA GCCCAGCGTG AAATCGGCTT GGCGGCAGGC GAGCCGCCGA  
21151 CGCGGCGCGG CTATCCACCG TCGGTGTTCTG CCACCTTGCC CAAACTGATG  
21201 GAGCGCGCCG GCATGAACCA GACGGGTTCG ATCACGGCGC TGTATACGGT  
21251 GCTGGTCGAG GGGGACGACA TGAACGAACC GGTGGCCGAC GAGACGCGTT  
21301 CGATACTGGA CGGCCACATC GTGCTCTCGC GCAAGCTGGG AGCGGCGAAT

Figure 5 (continued)

21351 CACTATCCTG CCGTCGACGT GCTGGCCTCG GCCAGCCGGG TCATGAATGC  
21401 CGTGGTGTCTG CCGCGTCACA AGTACCTGGC CGGACGTATG CGCGAACTGA  
21451 TGGCCAAGTA CCAGGATGTC GAGCTGTTGG TGAAAATCGG CGAGTACAAG  
21501 CAGGGCGCCG ATGCGTCGAC CGATGAGGCG ATACAGAAGA TCGGACAGAT  
21551 CAATGCGTTT CTCAGACAAC TAACCGACGA ACGCGAAGCA TTCGAGGATA  
21601 CCGTACTGCG CATGGCTGAA ATCATCGGAC CCGAATCCTA ATGGACCTGG  
21651 AAAGCCTGCT TGCCATCAAG CATTTTCGCG CCGACCAAGC CCAGCTTGCG  
21701 CTGAAACGCC AACAGCAGGC CTGCGCGGTT GCTGCCGCGG CGCAGCGTCA  
21751 GGC GCAAGGC CGCCTCGACG ATTGTCGCCT GTGGGCCGGA CAGCTCGAAA  
21801 ACCGTCTATA TGCCGAGCTG TGCCGGCGCA TCGTCAAGAC ACGCGACATC  
21851 GACGAGGTGC TGCAACGAGT GGGCCACGCC CGCGACCGCC AGGCCAGCCT  
21901 GGC GCTGCAG CTCGACGACG CCGTGCGCCG TCACGAACAT GAAATCCAGC  
21951 TGCTCGCGCA GCAGCGCGAG CAGCACCGGG AGTGCTTCCA GGC GAGCAA  
22001 CGGATCGCCG AGTTGGTGCG CCTGCAGCAG GTCGAGGCGG CGGCCTTGCG  
22051 CGAGAGCCAG GAAGATCGCG AAATTCAGGA AGCCATCGAA TTGTCGGCGC  
22101 GTGGGCGCGA CGATGCATCG CGAGCCGGCG ACGGCCTGGC GCGGCTATGA  
22151 ACCAGCCAGA CGGGCTGGGT TCGCCCATGG CCGGCGGCGG GCAGCGCATG  
22201 GCGGTGGCGC GCACGCCGTA TGCGCGTCAG CCGGATCGGG ATGCGCAGCG  
22251 TGCCCTTCGAG CGGGAAATGG AACAGGAGAA AGCGAAGGAA GAACTGCCCC  
22301 GGCCGCAACG CCTGGCGCCG GGTCCGGCCT GCGTCGGCTG GCTGGCGTCG  
22351 ATGGAACCTG CCGCCGGCCG TCCACCGGCC AGTCTGGCCC AGGCGCTGGC  
22401 AAGCGTGGCT GCGGGGCTGG CGGTAGGCGA CGTGCTGGAG GGGTATCGCG  
22451 AAGCCCGTAT CGTTGTGGAC GATACGCTGC TACCCGACAC CACCTTGTCTG  
22501 GTACGGGAGG ACGGCGGCTG GATCGTGGTG GCTTTCGCAT GCCGACAACG  
22551 GGACGCTTGC GAGCGCCTGC ACGCGTGCGC CGACCGGTTG GCCATGGAGC  
22601 TCGCGCTGGA GCTGGCGCGC GACGTCGAGG TTGCGGTGGC ATGCGACGGC  
22651 GAGCCGCACG AGCGGGTGGC GCGCGCGCAG CGGCCGTGGC GATGAATCGA  
22701 GTGGCCGGCG GGGCGGCGGC GCAGGCCGCT GGCATGGTGG ATCTCGCGGT  
22751 TCCGCGGTTG AGCGCCGGCG AGGCCCATGC CCTGTCGAGG ATTGCATGCC  
22801 ATGGCGCGCG ATTCGACGTT CGGCTTGCGG AGCCGGCCGT GCGCTGGCAC

Figure 5 (continued)

22851 TGC GCCCTGA CGCCTTGCGT GCACGGCGAC CTTGCCGATG GCGAGATGGA  
 22901 AAGCCTGCAA CTGCAATGGG CCGGGACGTA CATCGGCCTG ACGGTTCCGC  
 22951 GCGCGGCCGC GCGGGGATGG CTGGCGGCGC GCCTGCCCCG GTTTTCCGGC  
 23001 GTGGAGTTGC CGGAACCCAT TGC GGCGGCG GCCCTGGAGG CAATGCTGGA  
 23051 GGAGGTCTGT CGAGGCGTGG CCGGACTCGA CCAGCAAGGC CCGGTCCGCG  
 23101 TGGCGCGGCA AGGCGGGACG CCACCGGTCC AGCCGCATCG CTGGACCCTG  
 23151 ACGGTACGGG CGCCTGACGG TGGCGTCTGG CGCGCGGTAC TGGCGTGCGA  
 23201 CGCATGGGCC TTGCAAGCGG TCGCGGCGGC GCTGGATTCC GTTGCGCCTG  
 23251 CCGATGGTCG GGTCAATCCG GAGCGCGTGC CGGTCAGGTT GCGTGCCGAT  
 23301 GTCGGCGCGG CGTCCGTGAC CGCAGGCCAG CTGCGGACGC TCGAGCGGG  
 23351 CGACGTCGTG TTGCTCGCGC AGTACCGGGT GAGCGATGCC GCAGAACTAT  
 23401 GGTGTGTCGGC CGGACCCAGC GCGATCCGGG TACGGGCCGA GCATGCGTCT  
 23451 TTTCGTGTAA CTCAAGGTTG GACTCCCATC ATGACGGAAC CCGCGACACC  
 23501 TGACCCTGGC GAAACCCCGG CACAGGCCGA CGCGACGCTC GATACCGATC  
 23551 AGATACCCGT GCGCCTGACG TTCGACCTGG GCGAGCGCGA GTTCACGCTT  
 23601 GCGCAGCTGC GCAGCCTGCA TCCGGGCTGC ACGTTCGACC TCGAGCGGCC  
 23651 CATCGCCGAC GGGCCGGTCA TGGTGCGGGC CAATGGCCTG TTGCTGGGCA  
 23701 GCGGCCGGCT GGTGACATC GACGGCCGCA TCGGCGTGGT ATTGCAGTCG  
 23751 GTCAGGCCCTG GACTCGCATG AGCGATACCG ACCCCTTCAG CCTGGCCCTG  
 23801 TTTCTGGCGC TGCTGGCGCT GGTACCGCTC ATCGTCGTCA TGACCACGTC  
 23851 GTTCCTGAAG ATCGCCGTCG TGCTTGCCCTT GGTGCGCAAC GCCCTGGGAG  
 23901 TGCAACAGGT ACCGCCCAAC ATGGCCCTGT ACGGGCTGGC GCTTATTCTT  
 23951 TCCGCGTATG TGATGGCGCC GGTTCGTTAC AGGATAGGCA CCGAGGTCCA  
 24001 GGCCTTGACC GCGCAAGCCG GGGAGTCCGG CACCGCCGCG CCGATGGCGC  
 24051 TGGACGCCGT GCTTGCGGTG GCCGAGCGAG GCGTGGGGCC GCTGCGGGCC  
 24101 TTCATGTTGC GCAACAGCCA GCCGGCCCAG CGTGATTTCT TCCTGCGCAC  
 24151 AGCGCGTCAT CTCTGGGGCG AGGAGGCATC GCGGGACCTG TCGGAAGACA  
 24201 ACCTGCTGGT ATTGACGCC GCATTTCTGG TTTCGGAGCT GACCGCCGCA  
 24251 TTCCAGCTTG GCTTTCTGCT GTACCTGCCG TTCATCATCA TCGACCTCAT  
 24301 CGTATCGAAC ATTCTTCTTG CCATGGGAAT GATGATGGTT TCTCCCGTGA  
 24351 CGATCTCCAT GCCGTTGAAG CTGTTCCCTGT TCGTCATGGT GGACGGCTGG



Figure 5 (continued)

25901 TTGAAGAATA TCTTCTCGGC GCGCAACCTG ATGGAGTTCA TCAAGTCGGT  
 25951 ATGCAAGATC CTGTTTCTGG CGGTGTTGGT CACGTTGGTG ATACGGGATT  
 26001 CCTTGCAGCC GCTGATGGCC GTTCCCCATA GCGGGCTGGA CGGGTTGCGA  
 26051 ACGGGCGTAG GCCGCATTCT GCAGGTCATG GTCTGGAACA TCGGACTGGC  
 26101 GTACGGGGCG ATTTCTGCTGG CGGACCTGGC CTGGCAGCGT TACCAGTATC  
 26151 GCAAAGGCTT GCGGATGAGC AAGGACGAAG TGAAGCAGGA GTACAAGGAG  
 26201 ATGGAAGGCG ATCCCCATAT CAAGCAGCAA CGCAAGCACC TGCACCAGGA  
 26251 GCTGATCATG CATGGCGCGG CGGCCAGGT TCGCCGGGCG ACGGTGCTGG  
 26301 TGACCAATCC GACACACCTG GCCGTGGCCC TGTACTACGC GCGGGGCGAG  
 26351 ACGCCCTTGC CGCGCGTGCT GGCCATGGGG CAGGGAGCCG TGCCCGCTCT  
 26401 CATGGTCGAG GCCGCGCGCG ATGCCGGCGT GCCGGTCATG CAGAACGTCG  
 26451 CGCTGGCCCG CGCCTTGAC GACCAGGCGG AGGTGGACCA ATACATTCCC  
 26501 GGCGAGTTGG TGGAGCCGGT GGCCGCGGTG TTGCGGGCGG TCGCCAGGC  
 26551 ACTCAAGGAG CAGACATGAC AGCAACCATT CATCCCGATA TTGCCGATTA  
 26601 TGCGCGACGC CATGGCCTCG AACCTCGGT CGACGCCGAT GGCGGGCTTG  
 26651 CCGTCCGGAT CGACGGACGG CATCGCGTCA GGTGATCCC CGCCGAAGAC  
 26701 GGCATGCTGG TGTTGCGGGC GCGGCTGGCC GAGCTGCCCC ATGGGTGGCA  
 26751 GGCGCGCGCG GCGCAGTTGC GCCGGGCGGG CCTGCTGGCC AGCGCCATGG  
 26801 CCCCTGCGAC CGATGCGTAC TGCGGCATAG ACCAGGGCGA AACCGCGTTG  
 26851 TATCTGCACC AGCGCGTCGC ACCGGCCGGC AGTGCCTGG CGGTGGACGA  
 26901 GGCGGTGGGC GAGTTCGTCA ATGCCTTGGC CACTTGGAAG AGGGCGATGG  
 26951 CGCAATGGCA ATAGGTCGGC TTGGGTATCT TGTCCGCGGC GCATGGGCCG  
 27001 GGGGTGTCAT GCTGTTGGCG GCCGGTAGCG CCTGGGCGGC GCCGAAGTGG  
 27051 CCTTTGGCGC CGTATAGCTA CTACGCGCAG CAGCAGAGCC TGTCCGATGT  
 27101 GCTGCGCGAG TTCGCCGAG GCTTCAGCCT GGCCTTGCAA CAGGGCAAG  
 27151 GGGTGCAAGG CGTGGTCAAT GGGCGTTTCA ATGCGCGCAC ACCCACGGAG  
 27201 TTCATCGAGC GTCTCAGCG CATCTATGGG TTCAACTGGT TCGTGCATGC  
 27251 CGGCACGCTG TATGTCAGCC GCACCAGCGA CGTGGTTACC CGCGCGGTGG  
 27301 ATGCAGCCGG CGCTTCGCCG TCGGCGTTGC GCCAGGCCTT GCTGCAACTG  
 27351 GGCATCCTGG ACGAACGCTT CGGATGGGGA GAGCTGCCGG CGCAAGGCGT  
 27401 GGCCATGGTG TCAGGGCCGC CGGCCTATGT CGCGCTGGTC GAGCAGGCGG

Figure 5 (continued)

27451 TAGCGGCGTT GCCCAAGGGG GCCGGCAATC AGCAGGTGGC GGTGTTTCGC  
 27501 CTCAAGCATG CTTCCGTGAG CGACCGGGTG ATCCGTTATC GAGACCAGCA  
 27551 GGTAGTTACG CCGGGGATGG CCACCATGCT GCGCCAATTG ATCCTGGGGG  
 27601 CGGGGCCGGG CAACGACGCG GCGCTGGCCG CGGTGGCGGC GCCGCTGCGG  
 27651 GAAAATCCGC CGGTGTTTCGG CGATGCGGCA GCTGACGGGA ACGCGCCGCT  
 27701 CGCTGGCGCA GCCCAGGCAG CCGGCCGGCG CCTGAGCGAG CCCAGCGTGC  
 27751 AGGCCGACAC GCGCCTCAAT GCCTTGATCG TGCAGGATAT TCCCGAACGG  
 27801 ATGCCAATCT ACCGTGCCCT GATCGAGCAG TTGGATGTGC CCAGCACCTT  
 27851 GATCGAAATA GAGGCCATGA TCGTGGACGT CAATACCGAT CTGCTCAACG  
 27901 AGCTGGGTGT CACCTGGGGG GCGCAGATCG GAACCACCAG CCTGGGCTAT  
 27951 GGCGATCTGG GGCTGCGTCC CGGCAACGGC CTGCCCCTGG ACGGCGCGGC  
 28001 GGCCGACCTG GCGCCCGGAA CCTTGGGGAT CAGTGTCAGT ACCCGGCTGG  
 28051 CGGCGCGCTT GCGTGCCTTG GAGTCGGACG GGCAGGCCAA TATCCTGTCT  
 28101 CAGCCGTCCA TCCTGACCGC CGACAACCTC GGCGCCATGA TAGACCTGTC  
 28151 GGATACCTTC TACATTCGCA CCCTGGGCGA GCGCGTAGCG ACAGTCACGC  
 28201 CTGTCACGGT GGGTACGTCG TTGCGTGTGA CGCCGCGCTA TATCGCCGCC  
 28251 AAGGGAGGAC GCCAGGTGGA ATTGGCGATC GATATCGAGG ACGGACGGGT  
 28301 CTTGCAGGAG TATCCCATCG ATGGTCTGCC CCGGGTTCGG AAAAGCAGCA  
 28351 TCAGCACGCT GGCGGTGGTG GGGGACGAGC AGACGCTGCT GATCGGCGGC  
 28401 TACAACAATC GCCGTGACGA AGAGCAGGTC GAGAAAGTGC CGCTGCTGGG  
 28451 AGATATCCCC GGCCTGGGGT TCTTGTTCTC GAGCAAGTCC CGGGCGGTAC  
 28501 AGCGCCGCGA GCGGCTGTTC CTGATCCGGC CGCGTGTCTG GGCTATCGAG  
 28551 GGCAAGCCGG TCTTCAGCCC CGTTGCGGGC ACGTCGCAGG TGTTTCATGAG  
 28601 CACGGGTTGG GGCGGGCATG GCAGCAGCCT GAGCATTGCA CCCGGCGAGG  
 28651 GCGGGCATAA ACAAGTGCGT CATGATGCCC GGGCGGGCAG GCCGGTCCGG  
 28701 CTGGTGCCGG ATTCATTGCA TGTGGAGTAT GGCGAGGCGG GGGAGGCGTC  
 28751 GCCCTGAGCG TGGCCCGGGC AGGGGGGCTA CGGCACGCTG TCGTAGCCTC  
 28801 GTTCGCGCAG CGCTTCGCGC AAGGCACAGC GGGCGCGGGA AAGTCGGCTG  
 28851 CGAATGGTTC CTACAGGAAC CGAAAGCAGT GCGGCAGCCT CTTCATAGGA  
 28901 GAGTTCTTCC ACGCCGACCA TGAGGATCAC GTCGCGCATG CTTTCGGGGA

Figure 5 (continued)

28951 GCTGCTCCAG CGCTTCGCGT AGCAAGCGCA TGC GTTGACG CTGCTCGGTC  
29001 ACGGCTTCGG GGTG GGC GCG ACTGCATGGC ATGACACCCA GGCTGGCGTC  
29051 GGAGGTGAAT TCATAACGGC GCTCTGGCGC ACGCGACAAG TGATTGCGGA  
29101 CCAGATTGAG CGCGATGCCG TACAGCCAGG TGGAAAGCTG GGAGTCGCCA  
29151 CGGAACGATT GATACGCGCG CGCCGCCTCG GCGAAAGCCT GCTGCGCAAG  
29201 GTCCTCGACG TCGCTGCTGT GGCCGATGTG CTTGGCGATG AATCTCTGTA  
29251 ATCGGCCGGC ATGTTCCGCT ACCAGGCTGC TCAGCAACTG CTGGTCCGAT  
29301 CGCTCGGTGG CGATATCGGG ATATGCGTTG TCCGGTTTTT CGAGAGAAGC  
29351 GGGAAGACCG GATGACGGGG GAGAAACCAT GCAAAGCGAT ACCAAGTGAA  
29401 AGGGTGATAA TTCACGTCAC CAAGATACTG ACTGCCGTT TTATCCGGCA  
29451 GTTGTTAACT TCCGAAACTA ATGTCGGATC GCGGTCGCTA CCGGAGCATT  
29501 CAGATACAAC GCGCTGAACG TCTTCCGTAA AACTTACGAC GCGACGTATG  
29551 GGGACTACAC CAGGTGGTGC GCAAGGACCT CGCGCAACCA TTCTTGCGCC  
29601 GTATGGGCGG ATACCGGCGC CTTACCTGAT TGTGCGGCGG ATGCGAAGCG  
29651 CTGCTGCATG CTTTCGTCGA TCGTGGGCGG CAACGTTCCG GATATCTGAT  
29701 CGGGAGAGAC ACCGGTGTGCG TGACAGACCT GTCGGAAGCC GGCGGCGTCG  
29751 GCGCGGGCCG ACCAGGACAG GAAGGTAAGG AAATCCACGC CCTGCAGGGC  
29801 GTGGCGTGAG GTTTCCGCCA TGTCGACCGC TCGCGTGACG ACGCGCGCCG  
29851 ACAGCGATGT CGTCGGGACC GCGTGCAGAT CGAGCTCCTT GGCGACGGCG  
29901 CGGGCGATTG CCGTGCCGTA GGCCAGCGCC AGCGCGCTGG AAAACATGAC  
29951 AAGCGTGTCC TCGTCGGTGG CGACCCAGGA TACGTTGCGC CCTGACGGCG  
30001 TCGTGCCGGA TGCGATGACC TGGAACTGCT CGCCTGCTTT GGTGACATAT  
30051 AGGGTCTCTC CTTGGCTGGC GGCGCGCGCA AAGACATCAA GCTCCAAAGC  
30101 AGGTAAAGCG GGTGGGATCT GGAAGTTCAT GCGGTGGGCC GTTGTCTCGA  
30151 ATCCTTGGGT GTATCGCGTT CTACGGAGCG GGAACATGGA ATATGCACTG  
30201 TGTGACGCTT TCGGCTCTTC GGTTCATGC CGGCATGACA AACCCGACGG  
30251 CATGCAGGCC TCGTCCCGT CCGGCCACCG AGGCTCGCCT TGAACGCTGC  
30301 GCAAAGTGTC GGCCATACGC TGGGCCTCGA GCTGGAAATG GTGGTGGCCT  
30351 GTCGTGCGAC CGGGGCCAGC CATCCGGTGG CGCGCCATTT CGAAGCGCTG  
30401 CGCAACTTGC GCCGTCAACG CGGAGAGTCC GTGCAGGAGT ACCGTCTGGA  
30451 CGCGCGCCTG TCGGGGGGGT GGGCGGTCCC CATGGCCTGA GCGGGCTTGA



Figure 5 (continued)

30501 TAACGGCTAT AACCTGCTCG AAACCGCATT CGCGCCTGTG AACGGCGGCG  
30551 CGGGAGGGCT GCGCCGACTG GCCGAAGCGG TGCGCCGCGA GCTGCGTGAC  
30601 ACGCAGCTCG CCCTGGCCGC GGAGGGGGCG ATGCTGATCA ACGCGGCCGA  
30651 GCACCCCGCC GCCAGCCTGG ATGCCGACTG GTACCGGCGA GTGCGGGTGC  
30701 CGCGGCCCAT CTACGAGGAA CTCGTCGGCC AGCGAGGCTG GCTGCACCGG  
30751 ATCGGGATAG ACGCCAAGGC ACAGAACAGC CCCTGCACGT CGGTTCCCGT  
30801 GGCCATCGCC GCGCGCTGCC TGAACGTCGT GCTGGCGCTG GCTCCCGCGC  
30851 AGATCGCCAT GTTCGCCAAC AGCCCGCTGG AGGCAGGGCG GGTGACCGGT  
30901 CTCAAGGAAA ACCGCCTGAC CCTGTGGCCG CGCATGTTCC GAGCGCGCGC  
30951 CTACCTGGGC GACGACCTGC TGCATCGCCT GCCTGCAAGG CCGTTTCGCG  
31001 ATCTCGGCGA TTATTTCCGC TGGATGTTCG GCGGATTGAC CGCCAGCCGG  
31051 GCGCTACCGC CGGGCGACGC TTGCGACTAC AAGAACGCCG ATGTGGCCTG  
31101 CCTGGTGGGA GCCCCTTCGC TGGCAGAGTT CCTGTATGCG GGC CGTGGT  
31151 CCGCGCGAAA CCTGAATGAT GGCGGTTCG TCGTCTGGC CGCGCGCAGC  
31201 GAACATTTTCG TCTATTCGCA GTTCGCGCAG TTCCTGGACG CGCGTTGGCG  
31251 CTACAGGATG CCGATTGTCC CCGCCTTGCC GGCCTGTGTTG CGAGCCTGGG  
31301 ACAGGCAGGG CGGCCTGGAA GCGCTGTTCG AGCAGGCCGG CGCGCAAGGC  
31351 TACATCGAGG GGC GCGCGCC GGGCGCGGTA TTTGCCGATG CCGACTTGCT  
31401 GAGCTCAGCC GGC GATGCAG TCGCGGCCAG TGCGCCGATG GCGCGCTCGG  
31451 CGCTGCAATT GGGGCTGTTG CGCAATCTGC ACGACGCCGA GGCCCTGTTG  
31501 AGGCGATGGG GCTGGCTGCG CTTGCGTGCG TTGCGCGATC GGGCCATCGC  
31551 TTTGGCGTTG GACGATGCGC AGGTGCGCTG CCTTTGCCAA CAGGTCGTGG  
31601 CGGTAGCCGA AGGCGGGCTG GCCGGCGACG AGCAGCAATG GCTCGATTAT  
31651 GTGCGTTACG TGGTGAAAC CGGCGAGACC GCCGCGGACC GCATGCTGCG  
31701 CTTGTGGCGC CAGGCGCGCG GCACGCTGA GATGCGCCGC GCACAGGCGT  
31751 GCCGGCAGCG CGCGGTGCTG TCCTAGGATC CGGCACATTC CTTGCCAGGT  
31801 CTCGTTGGCC AGGCCTGTCA GTGCTCCGCT TCGTACACCT CGTCGGCCGC  
31851 CAGCAGAATA TCCACCGGTG GGTGCAAGCC GATGCGCCGC GCCGTTTCCA  
31901 GATTGATGGC TATCTTGGCG GGGGCATTCC AGACCTGGCT GATGCTGCGC  
31951 GGCTTTTCCC CATTGAAAAT GCGGGCAATG GTCTGGGCGT GGAACATGCC

Figure 5 (continued)

32001 TACGCTGGAG TAGTCCGCCT TGGCCAGGCT CATCAACAGG CCGGCCTTGA  
32051 CCTCGTCGGA GCCTTGATC GAGAACTCG GCACGCGGGC GGCGCGCAGC  
32101 AGCGCGGCGA GCTGCTTGAC GGACGTCGAG GTGATGCCCC GGTGCTCGGT  
32151 GACGTAAAAG GCGTCGACTT CGCTCGACAG CTTCTGGTAG CAAGCCAGAA  
32201 CGTTCTGGGT TGCCGTGGCG ATGGGGATGC CGGTGCGCGG TCGCTCGCAA  
32251 CGCTTGACGG AGAAATCCAA TGCCGGCATT AGTGCGGCGA CTTATCGAT  
32301 GGCTGCGTAG GTGCGACCTG CTTGCGGTGTC TTCGTAGACC AGTCCAAGCG  
32351 TCTTGAACGG CACGATGTCA TGGAGCAGCT GGATCTGCCG CTGGTAGTGG  
32401 TCGGGCTGTA CCCGGGCATG CAGGTTGTCC TGGCCGCTGT CGGCCGCACT  
32451 GGGTATGATC CGGGCGCTTA TCGGGTCGGT CGACGAGACG ACCACGGTGG  
32501 GTACCGGCGT GCCCAGTTCG ACCATGTCCT GTCCAGCCCA GGTACCCATG  
32551 GCGATGATCA GGTCGATGTC CTTGGCGCCA TGCAGGCGTG CCGCAACGGC  
32601 TTCGCGCACG GCAGGCCGCA AGGCGGTGTC GAAGTTGCCG GGCTGCCACC  
32651 ACGCATCGGG CACGAACCTCG ATGTAGTTGC TGCGGGCATG CGTGGCCAGG  
32701 TAAAGCCAGG CCTTTCGCAT ATCGGTTATC TCGGGCATGT CGTCGATACG  
32751 CAGCCATCCG AGTTGTTGCA ATGCGCGCGC GATCGCGTAG AGCGTGC GCG  
32801 GATACTCCTC GTACTCGCCG CTACCCACAT AACCGATGCG CCATTTCCGG  
32851 CCGGATGTAT GGGAGGGAGG CGGAAGGCGG GGCGAGGTAA GGGCGACGCC  
32901 GGAGCTCAGG GCCGCGACAG GAGGAGGGCT GGATGCCGCC GCGATGGGCC  
32951 ACGCGAGGCC AAGAAGCAGG GCGAGCGGGG CGAGTATTCC GGGGCGAAGG  
33001 GTCATGGGCG ATGAATGGCG ATGATGGTGA GATCGTCGGA TTGTTGCGAA  
33051 TCGGCGGCGA ATTCGCGTAG GTCGTGCAGA ATGTGCTCGA TGAGTTCGGC  
33101 CGCTGCGTGC GGCGCGCCCT GCATCAGGGC GACCAGCCGC GGCAGACCAT  
33151 ACTGGGCGCA GCCGCCGTGG ATGGCTTCGG TGACGCCGTC GGTAACGCG  
33201 ACCAGCGAGG TGCCGTTCGG CAAGGTGGTG CTCAGGGTGG AATACGCCTC  
33251 GTTGTCCAGC ACGCCGACAG CCGCGCCGCT GCTTCCTTGA AGCAGGCGGA  
33301 CCTCGCCACG TTCGTCGATG AGCAGCGGCG GCGGGTGGCC GCGTTGACC  
33351 CAGGCCAGGG CGCCTGTTTC CGGGGTGAAG ACGCCTATCA GCAAGGTGAC  
33401 AAACATCAGC TTGGGGTTGT TCTCGGCCAG ACGGTGGTTC ACCTGGTGG  
33451 CGATGGCGCC CGGGTCGTGC TCTTCTTCCG CCACGCTGCG TATCAAGGTC  
33501 CTGACGATGG CCATGAACAG GGCCGCGGGC ACGCCTTTTC CGGATACGTC

Figure 5 (continued)

33551 GCCGATGGCA AAGCACAGAC GCCCGTCTGC CAGCACGAAG TAGTCGTAGA  
 33601 AATCCCCACC GACCTCCCGG GCCGGGTACA TGACGGCACG CAACTGGCTG  
 33651 CCGCGCGTGG CCGCATCGGG CAACGGCTGG GGAAGCAGGC CAAGTTGGAT  
 33701 GGAGCGGGCG ATGCTCAATT CGCTTTCGAG GCGTTCGCGG TTCGATATCT  
 33751 GCGCCATCAG GGCCCGCACA TTGTGGTGCA GCTGTTCGTT CATGAACAGG  
 33801 AACGATTTCG CGAGCTGTCC GACTTCGTCC CGCCGCCGGC GCGGCAGGCA  
 33851 TGCCACCGAC GCGGGAACCC GGATCGGCTC GGTGAGGTCC TGGGTGGGAA  
 33901 GCTGGCGAGC GTAGTTGCTC AGTTGCGCCA ACGGCCGGGC GATGCGCACC  
 33951 GCCACCACCC ATGCCAGCAT CAGCCCGGCC AGCAAGGTGG CGGCGAAGAT  
 34001 CAGTGCCTGC CGGCGCACCA GATTCTGTGC CGGGTCGGTC AGGTCCGGCT  
 34051 CGGGAACGAC ACCGATGATG GTCCAATGCA GCGGCTTGTA TCGCAGGGCG  
 34101 TCGATCTGCC AGGCGCTTTC GCCGTTGGTA AAGCGCAACG TCAGGCCGCG  
 34151 GGTAGACGAG ATTTCGGCAA GCATCGAATG CAATACCCGT CCCGATTCCA  
 34201 CGTCTGTCTG GTCCAGCAGC CGGGCGGCCG ATGGGGGTGG CGGCACGATC  
 34251 ACCGTGCCAT CGTCCGCAAC CACGAACACG AAACCATGGC GGCTGAGCCG  
 34301 CAGCTCCGAC AGGTTCCGGT CTATCGCGGC AATCATGTTG GCTTCTGGG  
 34351 CGGCAACCTT GTCGATGATG GCTTGTGAGC TATCGGAGAT GGCGAGAACC  
 34401 CACTTCCACG CGGGGAAGTA CACGAAATAG GCGTGTCTCA TCTGGGCGGA  
 34451 CTCGTCCAGG GGAGACGGGT AGATGGCGAA GCCGCGACCG TCGTTGCGGC  
 34501 TTTCCTCGTA CATGGCGGCG GCGAGCGGCC GGCCCTTGAA GTCGCGGATC  
 34551 CCGGAGAGGT CCCGGTCGAT CATCCGGGGG TTGGTGCTGG CCAGCACGGT  
 34601 GCCTTCCGCG TCATAGGCGA AGGCGACGCG GCGCGGTCCC AGGTCGAGAT  
 34651 GGTTCAGCCA GACACGCGCC ATGCCCTTGG CGGCGCCGGT AGTGACGTGT  
 34701 CCGCGCTCGG CCTGCGCGGC ATAGGCGTTC AGCACCGATG TCACGACCGC  
 34751 GCTGAGTTGG ATCAGTTGCC TGCGGCTTTC CCGGATGGTG CGGATCTTGT  
 34801 CGTCGAGCAG CGTGGACCAG CGCGTGTGG TGTCACGAAC TACCAAGTCC  
 34851 ATGATGTTAC TGACGGCATG CAGTTCGTTC TTGATGATGT GTTCGTGAC  
 34901 ATCGCGCTGG GTCACGAGCA TCACGACGAT GCCTACGAGT AGCAGCGTGG  
 34951 ATGCAATGAG CAGGAGGAAC TTCCCACGCA ATGAAAGCGG CAACTCTAGC  
 35001 CGGGGAGTAC GGCGCATGAA CATGAA

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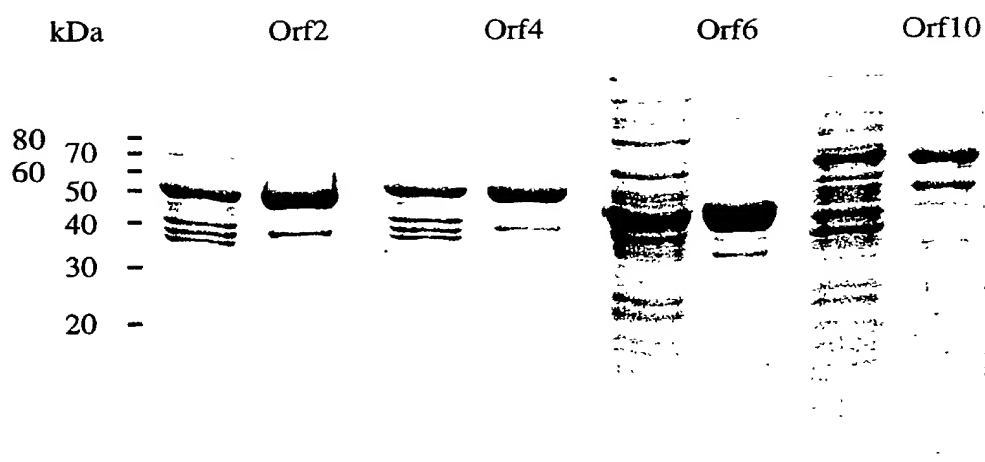


FIGURE 6

Docket No.: B45168

PCT/EP99/10297

## DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

VACCINE

the specification of which (check one)

☐ is attached hereto.

☒ was filed on 21 December 1999 as Serial No. PCT/EP99/10297  
and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or Inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

| Prior Foreign Application(s)<br>Number | Country       | Filing Date      | Priority Claimed |
|--|---------------|------------------|------------------|
| 9828217.1                              | Great Britain | 21 December 1998 | Yes              |

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

| Application Number | Filing Date |
|--------------------|-------------|
|--------------------|-------------|

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

| Serial No. | Filing Date | Status |
|------------|-------------|--------|
|------------|-------------|--------|

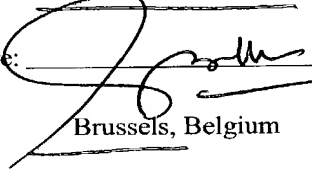
I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

Customer Number 20462.

Address all correspondence and telephone calls to Zoltan Kerekes , SmithKline Beecham Corporation, Corporate Intellectual Property-U.S., UW2220, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939, whose telephone number is 610-270-5024.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Date: June 14<sup>th</sup> 2007

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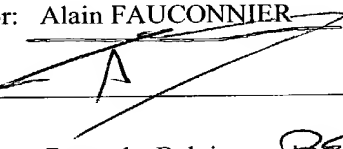
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